BAŞKENT UNIVERSITY
INSTITUTE OF SCIENCES
DEPARTMENT OF MOLECULAR BIOLOGY AND GENETICS
MOLECULAR BIOLOGY AND GENETICS MASTER'S PROGRAM

TRANSCRIPTIONAL PROFILING IN *ARABIDOPSIS THALIANA* *SLIM1* MUTANT EXPOSED TO BORON TOXICITY

BY

BEDRİYE NİHAN YURTSEVEN

MASTER OF SCIENCE THESIS

ANKARA – 2024

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This study, which was prepared by Bedriye Nihan YURTSEVEN within the framework of the Molecular Biology and Genetics Master's Program with Thesis in Molecular Biology and Genetics, was accepted as the Master's Thesis by the following jury.

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I would like to dedicate this study to my family for their love and support throughout this journey. Your trust in me has been a constant source of motivation for me and I am grateful for your presence in my life. Thank you all for your invaluable contributions and being a part of this research effort.

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ABSTRACT

Bedriye Nihan YURTSEVEN
TRANSCRIPTIONAL PROFILING IN *ARABIDOPSIS THALIANA SLIM1* MUTANT EXPOSED TO BORON TOXICITY
Başkent University Institute of Sciences
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The effects of B (B) toxicity on plants have been investigated for a long time, but the interaction between sulfur metabolism and B has not been sufficiently clarified in this process. Sulfur metabolism plays an important role, especially in the production of compounds such as cysteine, methionine and glutathione (GSH), and is considered a critical component in the stress response of plants. In this study, the effects of sulfur metabolism in response to B toxicity and the role of the SLIM1 transcription factor in this process were investigated. SLIM1 is an important transcription factor that controls the synthesis of sulfur-containing amino acids by regulating sulfur uptake and metabolism. The deficiency of this factor in *slim1* mutants leads to disruptions in the functioning of sulfur metabolism. In this study, the effects of sulfur metabolism were investigated in *slim1* mutant *Arabidopsis thaliana* plants exposed to B toxicity.To better understand the functions of sulfur (S) uptake and metabolism in plants at the molecular level, it is necessary to investigate these processes under the effects of B toxicity. For this purpose, in this study, wild-type and *slim1* mutant plants of *Arabidopsis thaliana* exposed to 1 mM and 2 mM B and RNA-Seq analysis were performed As a result of the analyses, significant changes were observed in genes related to sulfur metabolism and transport in the *slim1* control group. While a significant increase in ribosome biogenesis, RNA processing and binding activities was detected in *slim1* mutants under B toxicity, glucosinolate biosynthesis processes were suppressed in all *slim1* mutants. In addition, it was revealed that ribosome biogenesis and RNA processing processes, together with antioxidant mechanisms, play critical roles in the regulation of defense responses against B toxicity.This study provides important contributions to the understanding of the mechanisms of resistance to B toxicity and reveals that targeting SLIM1 and related mechanisms provides valuable clues for the development of plant varieties resistant to B stress conditions.

KEYWORDS: B toxicity, S metabolism, SLIM1, RNA-Seq, *Arabidopsis thaliana*

ÖZET

Bedriye Nihan YURTSEVEN
BOR TOKSİSİTESİNE MARUZ KALAN *ARABIDOPSIS THALIANA SLIM1* MUTANTINDA TRANSKRİPSİYONEL PROFİLLEME
Başkent Üniversitesi Fen Bilimleri Enstitüsü
Moleküler Biyoloji Ve Genetik Programı
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Bor (B) toksisitesinin bitkiler üzerindeki etkileri uzun süredir araştırılmakta, ancak bu süreçte sülfür metabolizması ve bor etkileşimi yeterince açıklığa kavuşturulamamıştır. Sülfür metabolizması, özellikle sistein, metiyonin ve glutatyon (GSH) gibi bileşiklerin üretiminde önemli rol oynar ve bitkilerin stres yanıtlarında kritik bir bileşen olarak görülmektedir. Bu çalışmada, bor toksisitesine yanıt olarak sülfür metabolizmasının etkileri ve bu süreçte SLIM1 transkripsiyon faktörünün rolü araştırılmıştır. SLIM1, sülfür alımı ve metabolizmasını düzenleyerek kükürt içeren amino asitlerin sentezini kontrol eden önemli bir transkripsiyon faktörüdür. *Slim1* mutantlarında bu faktörün eksikliği, sülfür metabolizmasının işleyişinde bozulmalara yol açmaktadır. Çalışmada, bor toksisitesine maruz kalan *slim1* mutant *Arabidopsis thaliana* bitkilerinde sülfür metabolizmasının etkileri incelenmiştir. Bitkilerde S alımı ve metabolizmasının işlevlerini moleküler düzeyde daha iyi anlamak için, bu süreçlerin B toksisitesinin etkileri altında incelenmesi gereklidir. Bu amaçla, bu çalışmada *Arabidopsis thaliana*'nın yabani tipi ve *slim1 mutant* bitkileri, 1 mM ve 2 mM B'ye maruz bırakılarak RNA-Seq analizi gerçekleştirilmiştir. Analizler sonucunda, slim1 kontrol grubunda sülfür metabolizması ve taşınmasıyla ilişkili genlerde belirgin değişiklikler gözlenmiştir. Bor toksisitesi altında slim1 mutantlarında ribozom biyogenezi, RNA süreçleri ve bağlanma aktivitelerinde önemli bir artış tespit edilirken, tüm slim1 mutantlarında glukosinolat biyosentezi süreçlerinin baskılandığı belirlenmiştir. Ayrıca, ribozom biyogenezi ve RNA işleme süreçlerinin, antioksidan mekanizmalarla birlikte bor toksisitesine karşı savunma yanıtlarının düzenlenmesinde kritik roller oynadığı ortaya konulmuştur. Elde edilen bulgular, Bu çalışma, B toksisitesine karşı dayanıklılık mekanizmalarının anlaşılmasına önemli katkılar sağlamakta ve B stres koşullarına dayanıklı bitki çeşitlerinin geliştirilmesi için SLIM1 ve ilişkili mekanizmaların hedeflenmesinin değerli ipuçları sunduğunu ortaya koymaktadır.

ANAHTAR KELİMELER: Bor toksitesi, sülfür metabolizması, SLIM1, RNA-Seq, *Arabidopsis thaliana*

PREFACE

This thesis titled "Transcriptional Profiling in *Arabidopsis Thaliana Slim1* Mutant Exposed to Boron Toxicity" aims to understand the molecular mechanisms by which plants develop under B toxicity and S deficiency. In our research, the responses of *slim1* mutants to B applications were examined in detail.

Nowadays, understanding abiotic stress factors affecting agricultural productivity is becoming increasingly important. B toxicity is a condition that occurs as a result of excessive B accumulation in the soil and negatively affects plant metabolism. S metabolism is a critical building block for proteins, coenzymes and antioxidants. In addition, S-containing compounds play an important role in the defense mechanisms of plants against abiotic stresses and in maintaining redox balance.

In this study, *slim1* mutant *Arabidopsis thaliana* plants were exposed to 1mM and 2mM B applications and gene expression changes were examined in detail by RNA-seq analysis. In addition to differential gene expression analyses, GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analyses were performed, and important findings were obtained regarding S metabolism and defense mechanisms on the stress caused by B toxicity. It was observed that B is effective in the synthesis and transportation of S-containing compounds under toxicity. These results provide a basis for future molecular and genetic approaches to increase B tolerance in plants.

I would like to express my gratitude to everyone who contributed to the realization of this study. First of all, I would like to express my sincere gratitude to my advisor Assoc.Prof. Dr. Ceyhun KAYIHAN, who guided me in shaping and completing this project. I would also like to thank Başkent University and Kayıhan laboratory for making this study possible and Asst. Prof. Dr. Emre AKSOY for his support. I am also grateful to my family and friends who supported me and believed in me throughout the study.

I hope that this thesis will make a useful contribution to better understand the plant's tolerance mechanisms to stress conditions such as B toxicity and S starvation and inspire new research in this field.

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ABBREVIATIONS LIST

ABA	Abscisic Acid
ABC	ATP-Binding Cassette Transporters
APK	Adenosine 5'-Phosphosulfate Kinase
APS	Adenosine 5'-Phosphosulfate
APX	Ascorbate Peroxidase
BOR1	B Transporter 1
CAT	Catalase
EBF1	EIN3-Binding F-Box Protein 1
EBF2	EIN3-Binding F-Box Protein 2
EIL	Ethylene Insensitive-Like
EIN3	Ethylene Insensitive 3
GO	Gene Ontology
GOT	Glutamate Oxaloacetate Transaminase
GR	Glutathione Reductase
GSH	Glutathione
GSL	Glucosinolate
KEGG	Kyoto Encyclopedia of Genes and Genomes
MDA	Malondialdehyde
MOHAR	Monodehydroascorbate Reductase
MRP	Multidrug Resistance-Associated Protein
NADH	Nicotinamide Adenine Dinucleotide (Reduced Form)
NADPH	Nicotinamide Adenine Dinucleotide Phosphate (Reduced Form)
NIPT	Nodulin-Like Integral Membrane Protein Transporter
OAS	O-Acetylserine
PERE	Promoter Element for Reactive Elements
PSII	Photosystem II
RGII	Rhamnogalacturonan II
ROS	Reactive Oxygen Species
SLIM1	S Limitation-Inducible Factor 1
SOD	Superoxide Dismutase
SULTUR	S Metabolism
TEBSS	Transcription Element-Binding Specific Site
UPE BOX	Ubiquitin Proteasome Element Box

1. INTRODUCTION

Boron (B) is involved in various physiological and metabolic processes in plants and affects plant development. It plays a role in numerous processes, from contributing to the structural function of the cell wall to carbohydrate metabolism. B binds with pectin in the cell wall structure, providing mechanical strength and stabilization to the overall structure. Some of the places where the element B is found include rocks, soil, rivers, oceans, and volcanic and geothermal sources. Due to the natural geological structure and climatic conditions, B toxicity or B deficiency can occur in some regions. This situation seriously affects crop yield and quality.

B regulates enzymatic or non-enzymatic pathways to reduce the effects of reactive oxygen species (ROS) accumulated under abiotic and biotic stress conditions. Plants develop some tolerance mechanisms under stress conditions. Components such as S-containing GSH and secondary metabolites especially play an important role in reducing the harmful effects of B toxicity. The interaction between B and S metabolism is also an important subject. B's function in maintaining the permeability and integrity of cell membranes is critical for the intracellular transport of S-containing compounds such as methionine and cysteine. In addition, it affects processes such as RNA processing and protein synthesis.

SLIM1 is one of the key regulators of S metabolism. However, there are other processes that SLIM1 affects and has a role in regulating. Apart from S starvation, it also functions to reduce the plant's responses to other stress conditions.

S metabolism synthesizes compounds that function as antioxidants and defense mechanisms in plants. These S-containing compounds play important roles as defense mechanisms in B toxicity. In particular, it is one of the important metabolites of glucosinolates, their production and defense mechanism against B toxicity. Understanding the effects of S metabolism on B toxicity at the molecular level will provide a better understanding of the tolerance mechanisms developed by plants under B toxicity.

2. LITERATURE

2.1 Boron (B) Element in Plants

B is a semi-metal element. It is found in the form of boric acid (H3BO3) at neutral pH. B has a missing p-orbital in its chemical structure. Therefore, it does not ionize because it does not give protons like other acids. Instead, it acts as an electron acceptor, that is, a weak Lewis acid. This structure allows B to bond with water molecules or OH- ions [1] [2]

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Plants require certain nutrients to grow, develop and reproduce healthily. One of these nutrients is B, which was first identified by Warington in 1923 [3]. This discovery was supported by molecular studies on the B requirement of the *Arabidopsis thaliana* mutant. [4]Today, it is recognized as a micronutrient vital for the growth of many plants.

B is a vital micronutrient for plant growth and development and plays a critical role in many metabolic and physiological processes. Research shows that B has many important functions, such as providing stability in cell wall structure, supporting nutrient transport and metabolism, and contributing to plant defense mechanisms. Some of the processes in which B is specifically known to be involved are: Cell wall structure, cell membrane stability and permeability, photosynthesis, RNA metabolism, phenol metabolism, sugar transport and carbohydrate metabolism, and hormones. It plays an important role in maintaining the structural integrity of the cell wall, especially required for the cross-linking of pectic polysaccharides. This increases the mechanical strength of the cell wall and supports its stability [5].

It also plays an important role in ensuring the functionality and stability of cell membranes. B interacts with the components of the cell membrane, thus regulating the membrane structure and permeability. This process is very important in terms of nutrient transport, signal transmission and cellular communication. This process contributes to the regular transport of carbohydrates, water and other essential nutrients within the plant. On the other hand, it supports membrane integrity by affecting the ion balance [6] [7].

B is also closely related to the stages of plant reproduction and growth. It is necessary for pollen viability, pollen tube development, fertilization, seed and fruit development. It is also important in the growth and development stages of roots [8].

B plays an important role in combating stress by reducing the effects of reactive oxygen species (ROS) formed in plants under abiotic and biotic stress conditions. This process is carried out through the production of antioxidant compounds, primarily glutathione (GSH). Glutathione is a S-containing compound that reduces the effects of ROS and provides cellular redox balance [9].

B's association with S metabolism supports the stabilization of cell membranes and ion transport. It regulates membrane integrity, intracellular and intercellular transport of S-containing compounds such as glutathione and methionine, and also contributes to metabolic processes such as RNA processing and protein synthesis [10].

The optimum content of B in plants is vital for this micronutrient to provide its beneficial effects. The appropriate B concentration for plants is generally found in a narrow range of 0.5-2 ppm. This range may vary depending on the plant species and growing conditions [11].

When this level is decreased, B deficiency symptoms appear. In case of deficiency, problems such as cell wall stability, growth retardation, lack of flowering and slow root development are observed. Deficiency also negatively affects reproductive processes such as pollen tube development and seed formation. If the B level exceeds this range, toxicity symptoms are observed. In case of toxicity, effects such as damage to cell membranes, chlorosis, necrosis and decreased photosynthesis capacity occur.The presence of B at appropriate levels ensures that the growth, metabolic balance and defense mechanisms of plants work effectively. Optimal B concentration is a determining factor in crop yield and quality [12].

2.2 Natural Sources and Distribution of B

Some of the places where the B element is found are rocks, soil, rivers, oceans, volcanic and geothermal sources. It shows a certain amount of distribution in these regions. Especially rocks and oceans are the largest B sources. It is known that the amount of B in rocks is 5-10 mg/kg and it is 4-6 mg/L in the ocean [13].

B is mostly trapped in rocks formation. Therefore, most soils can be considered deficient in B. In addition, the pH of the soil is a factor affecting B uptake. In neutral soils, the presence of boric acid (H3BO3) in the form allows B to be taken up more easily by the plant [14].

Precipitation and temperature are factors that affect the concentration of B in the soil. In humid regions with high rainfall, boric acid leaches from the soil and goes deep into the depths that roots cannot reach. For this reason, it is known that B deficiency in the soil is at a critical level in humid climates such as China, northwest India, Nepal, Japan, Bangladesh and Brazil. In addition, B deficiency has been observed in countries such as Zambia, Nigeria, the Philippines, Thailand, Korea and the Balkans, among others. In hot and arid climates, B can accumulate more on the soil surface and cause toxic effects [15] [16].

Apart from this, geothermal resources are also quite rich in B. In geothermal regions, water comes into contact with rocks and B dissolves and passes into the water. In hot and dry countries, water reaches the soil through capillary action. For example, it is known that geothermal waters in the Western Anatolia Region of Turkey are rich in B. This causes toxic B levels in the environment [17].

B toxicity has been reported especially in countries such as Australia, USA, Russia, Turkey, Mexico, Israel, Iraq, Egypt, Syria, Morocco, Jordan, Libya, India, Pakistan, Malaysia, Peru, Chile, Hungary, Serbia and Italy due to their natural geological structure and climatic conditions. (Ross O. Nable, 1997) Toxicity problems occur especially in the case of excessive use of B fertilizer or the presence of high B concentrations in irrigation water [18].

2.3 Functions of B in Plants

B plays a key role in many metabolic processes in plants. The main mechanisms that B is known to affect include nucleic acid metabolism, carbohydrate and protein metabolism, phenol metabolism, cell wall structure and stability, photosynthesis, ion transport, hormone metabolism, energy metabolism, redox balance, and response mechanisms to oxidative stress. Each of these mechanisms is critical to ensuring optimal productivity by directly affecting the growth and development processes of plants [19] [20] [21] [22].

One of the most important known functions of B in plants is to ensure the protection of the cell wall structure. In particular, its interaction with the RG-II (rhamnogalacturonan-II) molecule is very important for the cell. RG-II is a component of pectins in the plant cell wall. B interacts with the apiosyl side chain of the RG-II molecule to form a borate diester bond. In this way, it binds two RG-II molecules together and performs dimerization. This cross-linking forms the three-dimensional pectin network structure. Thus, it allows the formation of a tighter and more durable cell wall structure [23].

Apart from the RG-II molecule, lignin is a component that plays a role in the durability of the cell wall by binding to cellulose fibers. Lignin metabolism is known to play a role in water transport and resistance to stress conditions such as heavy metals, heat, and drought. (Liu Q, 2018) In the study conducted on citrus fruits, there was an increase in lignin synthesis under B deficiency conditions. This increase occurred especially in the leaf veins and excess lignin caused the veins to crack [24].

B has the ability to interact with diol groups. This property allows it to bind strongly to molecules with two hydroxyl groups. According to this chemical structure of B, there are potential targets that are expected to bind. These are the structures in the content of the cell wall, ATP, NADH, NADHP and ribose parts of RNA. These molecules are the main targets affected by B [25].

2.4 B Toxicity in Plants

B toxicity negatively affects photosynthesis and carbohydrate metabolism by binding to energy carrier molecules such as ATP, NADH, and NADPH. When B binds to these molecules, it limits the production of ATP and NADPH in the photosynthetic electron transport chain because it prevents these molecules from effectively using the energy they carry. Specifically, it reduces the production of energy molecules needed for the Calvin cycle by disrupting electron flow in components such as Photosystem II (PSII). Inadequate production of ATP and NADPH slows down the Calvin cycle, so the plant has difficulty converting CO2 into carbohydrates. Decreased carbohydrate production limits the plant's energy stores, slows growth rate, and inhibits synthesis of structural components.

This challenges the plant's ability to cope with oxidative stress, as ATP and NADPH are also important for antioxidant defense mechanisms. Therefore, B's binding and interference with energy molecules severely impairs photosynthesis and carbohydrate metabolism, weakening the plant's overall health [26] [20].

When plants are exposed to high levels of B, photosynthesis processes are severely impaired. The stress created by B toxicity in plants affects the structural and metabolic processes of photosynthesis through various mechanisms.PSII is a protein complex that is responsible for converting light energy into chemical energy. Excess B causes structural damage to this protein complex. This damage in PSII causes malfunctions even though it does not carry electrons and its photon capture capacity decreases. This reduces the efficiency of chlorophylls and affects the photosynthetic transport chain [27] [28].

Thickening was observed in young leaves due to B toxicity. As leaf thickness increases, internal CO2 transmission decreases [29].

In some studies, it is thought that B toxicity causes stomatal closure and this reduces the internal conductance of CO2. However, in this study, there was no change in stomatal conductance and the decrease in CO2 was attributed to the increase in the parenchymal layer [30].

Another factor affecting photosynthetic efficiency is the loss of chlorophyll. Oxidative stress caused by B toxicity causes the accumulation of reactive oxygen species and damages the cell. Increased malondialdehyde (MDA) levels were observed in the leaves of rice seedlings because of high B exposure. This was seen as chlorosis and necrosis at the leaf tips and resulted in a decrease in chlorophyll and carotenoid content [31].

Artemisia annua L. In the study conducted on plants, lipid peroxidase rates increased with B exposure and photosynthetic activity decreased accordingly. In addition, Methyl jasmonate hormone, known to regulate stress responses, was applied in this study. Improvement in photosynthetic activity and increase in antioxidant defense were observed in plants with B toxicity and Methyl jasmonate application [32].

In a similar study on sugar beet, B toxicity suppressed chlorophyll synthesis. It disrupted photosystem II (PSII) and the electron transport chain. Therefore, it caused a decrease in photosynthetic activity and plant growth. $ZnSO_4$ and MeJA applications were effective in preserving photosynthetic structures. It also caused an increase in antioxidant enzyme activities by reducing MDA levels [33].

2.5 B Tolerance

To achieve B tolerance, plants have developed various defense mechanisms over the years. First, plants try to limit their uptake of B and prevent it from accumulating to harmful levels by excreting excess B through the roots. This process is vital for plants exposed to high B concentrations [34].

Transcriptional responses of different genes in response to B toxicity in Triticum dicoccum plant were investigated. ABC transporter proteins and aquaporins were found to be effective pathways in B tolerance mechanism. By taking part in B transport and water balance, toxic effect on the plant was reduced [35].

Another study showed that BOR1 and NIP5;1 transporters work to reduce B charge. BOR1 ensures that excess B is removed from the root via xylem. Thus, it helps regulate B levels in the plant by protecting root cells from B toxicity. Furthermore, the activity of BOR1 is suppressed by endocytic destruction, limiting excessive B transport. In NIP5;1, there is no such destruction mechanism, but lateral diffusion mechanisms have been observed. This is one of the strategies used by the plant against B toxicity [36].

Significant physiological and biochemical changes occur in plants with the accumulation of reactive oxygen species (ROS) under various stress conditions. This ROS accumulation negatively affects cellular functions through effects such as lipid peroxidation in cell membranes, structural deterioration in proteins and DNA damage [37].

In order to maintain a balance between ROS production and detoxification, there is a complex antioxidant defense system. This defense system includes enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR) and non-enzymatic molecules such as ascorbate, glutathione phenolic compounds, alkaloids and α-tocopherols. These compounds prevent ROS from damaging cell components, increasing the plant's survival under stress conditions [38].

Glutathione and phenolics are important antioxidant compounds in plants. For example, one study showed that phenols have the capacity to destroy radical species and reduce their toxic effects by chelating metal ions. Phenolic compounds neutralize reactive oxygen species (ROS) thanks to the aromatic rings and hydroxyl groups in their structures and thus protect the cellular integrity of plants under oxidative stress [37].

This study showed that polyphenols have significant antioxidant effects in some plant species growing in saline environments. A correlation was found between the amount of polyphenols and antioxidant capacity. Phenolic compounds are an important defense element in plants both in the fight against radical species and against heavy metal toxicity [37].

Therefore, phenolic compounds play a critical role in plants' ability to cope with oxidative stress. Anthocyanins, a subgroup of these compounds, have the capacity to form chelates with metal ions. This property allows plants to bind toxic heavy metals and reduce the harmful effects of free metal ions in cells. Therefore, anthocyanins are an effective defense mechanism to overcome both oxidative stress and heavy metal toxicity [39] [40]. (Naing & Kim, 2021)

It is known that anthocyanins generally form chelates with heavy metals. However, recent studies have shown that they can also form similar complexes with B, a metalloid. In purple-leafed basil (Ocimum basilicum) plants, anthocyanins have been shown to protect chloroplasts from oxidative damage under B toxicity, thereby increasing photosynthetic efficiency. This study shows that the stress caused by B in chloroplasts can be reduced by anthocyanins. It is thought that anthocyanin-B complexes limit the free movement of B ions and prevent B from causing further damage to cells [41].

Another study found that purple-leafed basil plants contained higher levels of ascorbic acid, glutathione, and anthocyanins under B toxicity. These compounds provide strong antioxidant defense against oxidative stress caused by B in plant cells [27].

Following the discovery of the ability of the metalloid B and anthocyanins to form complexes, the next assumption is the formation of the glutathioneyl-anthocyanin-metalloid (GS-A-M) complex. It is suggested that GSH-conjugated anthocyanins will temporarily bind to metalloid ions in the cytosol, preventing free ions from damaging plant tissues. In addition, it is predicted that the complexes accumulated in the vacuole during this process will be stored in multidrug resistance-associated proteins (MRP) and ATP-binding cassette (ABC) carriers and transported out of the cell [42].

In support, the tolerance mechanism of anthocyanins was investigated in *Arabidopsis thaliana slim1* mutants exposed to high B concentrations. Anthocyanin accumulation in leaves was observed in direct proportion to the increase in B toxicity. In addition, there was an increase in the gene expressions of transcription factors such as MYB75 and MYB114, which contribute to anthocyanin synthesis, and the carrier protein TT19.

Even in GSH deficiency, the chelation and transport mechanism of anthocyanins provided an alternative defense. These results indicate that anthocyanins play a direct role in the reduction of metalloid toxicity such as B [43].

2.6 Glutathione-glucosinolate-anthocyanin

Glutathione (GSH) is a tripeptide antioxidant consisting of the amino acids glutamate, cytsen, glycine. It plays important roles in critical stages such as antioxidant defense, detoxification, maintenance of redox balance, carbon and nitrogen metabolism, amino acid and protein synthesis in the cell. Additionally, GSH is an essential component of S metabolism.

S is critical for GSH synthesis because cysteine is a S-containing amino acid. Plants need GSH production to prevent oxidative damage and to ensure detoxification under various stress conditions, a process that is directly dependent on S uptake. In the event of S deficiency, GSH synthesis decreases, which negatively affects the stress tolerance of plants. (GRAHAM NOCTOR, 2011) (Noctor G, 2011)

Anthocyanins are pigments that give red, purple and blue colors to flowers, fruits and leaves in plants belonging to the flavonoid family. These pigments are effective in protecting plants against environmental stresses. While they prevent DNA and protein damage by providing protection against UV rays, they play an important role in maintaining cellular redox balance by scavenging reactive oxygen species (ROS). Anthocyanins, which increase resistance to stress conditions such as drought, salinity and low temperature, also contribute to the expression of defense genes by regulating metabolic signaling processes. [44]

Glucosinolates (GSLs) are secondary metabolites rich in sulfur that play an important role in plant defense. They are especially prevalent in the order *Brassicales* and *Arabidopsis thaliana*. [45]GSLs consist of a β-thioglucoside moiety, a sulfonate, and a side chain that differs depending on which amino acid they are derived from. They are divided into 3 groups depending on which amino acid they are derived from. These are alpha-glucosinate, indolic, and benzenic glucosinate molecules. The alpha-glucosinate group is derived from the amino acids alanine, valine, leucine, isoleucine, and methionine, while the indolic group is derived

from tryptophan and the benzenic group is derived from the amino acid phenylalanine or tyrosine. [46] [47]

In order for GSLs to become functional, they must be hydrolyzed by myrosinase enzymes. As a result, reactive aglycones are formed, which spontaneously transform into toxic compounds such as isothiocyanates, nitriles and thiocyanates. These compounds play a role in plant defense by creating a toxic effect against insects and pathogens. Sulfur is an important nutrient because it is both a part of the structure of glucosinolates and a necessary element in their biosynthesis. Therefore, GSL and S metabolism are closely related [48].

Glucosinolates (GSL) and anthocyanins are secondary metabolites of critical importance in the stress response of plants, and both are linked to the phenylpropanoid pathway. Common compounds and enzymatic pathways are found in the synthesis of these molecules. In particular, glucosinolates and anthocyanins derived from aromatic amino acids such as phenylalanine and tyrosine play an active role in the defense of the plant against both biotic and abiotic stresses. In the phenylpropanoid pathway, the enzyme phenylalanine ammonia lyase (PAL) produces precursor compounds for both lignin and flavonoid synthesis, while one of the side branches of this pathway supports glucosinolate biosynthesis [49].

Under stress conditions, a metabolic switch between these pathways may occur, allowing the plant to optimize its defense strategies according to environmental conditions. [50] For example, anthocyanin production increases under oxidative stress, while glucosinolates accumulate under biotic stress. Both metabolites contribute to maintaining the redox balance in plant cells, preventing cellular damage and increasing the plant's chances of survival. Thus, GSL and anthocyanins form a complementary defense network, both directly and indirectly. [51]

GSH is another important component of this defense system and interacts with GSL and anthocyanin metabolism in various ways. As a powerful antioxidant, GSH plays a critical role in maintaining cellular redox balance and also acts as a precursor molecule in glucosinolate biosynthesis. While increasing the capacity of anthocyanins to combat oxidative stress, the role of GSH in detoxification mechanisms strengthens the plant's resistance to abiotic factors such as heavy metals and oxidative stress. Thus, GSL, anthocyanin and GSH increase the plant's adaptation to stress conditions and its chance of survival through complementary mechanisms. [52] [53]

2.7 Sulfur(S) Assimilation and Transport

S is a macronutrient required by all organisms. Plants use S as a source of sulfate and assimilate it into various organic S compounds. Animals cannot assimilate sulfate. Therefore, they must obtain the amino acids found in the structure of sulfate through their diet. From this perspective, plants play a very important role in the S cycle. Plants are the primary producers of organic S compounds. Therefore, they play an important role in the S cycle [54].

S is used in many important biochemical processes in the cell. For example, it is a part of the structure of important antioxidants such as glutathione and thioredoxin. These play a critical role in maintaining redox balance by regulating electron transfer during energy production and metabolic processes [55] [56]. In addition, the S-containing amino acids cysteine and methionine are used in protein synthesis and participate in the formation of disulfide bonds, which play an important role in stabilizing the three-dimensional structure of proteins. S is also found in the structure of coenzymes and vitamins such as coenzyme A, biotin, thiamine and lipoic acid, which allows it to play critical roles in energy metabolism and enzymatic reactions [57].

Plants uptake sulfate through sulfate transporters (SULTRs). After sulfate is absorbed by root cells, it is stored in the vacuole or enters the assimilation pathway directly. Sulfate assimilation is reduced by a two-step pathway. Both pathways share adenosine 5'-phosphosulfate (APS), which is activated by ATP Sylase (ATPS). The amino acid cysteine is the first product of primary S assimilation. (Carmen Rotte, 2000) APS is then reduced to sulfite (SO_3^{2-}) by APS reductase (APR). Sulfite (SO_3^{2-}) is then reduced to Sulfide (S^{2-}) by Sulfite reductase (SiR). Sulfide (S^{2-}) combines with O-acetyl-l-serine (OAS) to form cysteine. Cysteine is then converted to methionine and glutathione (GSH) [58] [59].

In secondary S assimilation, APS is phosphorylated to PAPS by the enzyme APS kinase (APK). PAPS is responsible for the formation of sulfated secondary metabolites formed by PAPS sulfotransferase (SOT) enzymes. Sulfated plant metabolites include glucosinolates, flavonoids, phytosulfokines, brassinosteroids, sulfojasmonate, and hormones [60] [61].

In a situation where plants face S starvation, they provide sulfate ions through their roots. In this process, carrier proteins in the membrane of plant root cells also contribute to the efficient transport of nutrients such as S. The SULTR gene family encodes carrier proteins responsible for the transport of sulfate. This gene family is divided into 5 groups according to their functions, and SULTR1.1 and SULTR2.1 are the most critical members [62].

SULTR1;1 is a carrier protein that acts as the first step in a stress condition such as sulfate deficiency. This carrier protein shows increased expression under sulfate deficiency conditions and provides sulfate uptake with high affinity. It is expressed more especially in young root tissues, allowing roots to effectively uptake sulfate from the soil. SULTR21;2 is involved in the transport of sulfate at lower levels [63].

The SULTR2 group also shows increased expression in sulfate deficiency and is involved in low-affinity sulfate uptake. SULTR2;1 and SULTR2;2, unlike the first group, ensure that sulfate taken from the roots is transported to the organs. At the same time, when there is an increased toxicity in the plant, it effectively distributes sulfate to the organs. Thus, it allows the transported sulfate to be used for assimilation [64] [65].

2.8 S Deficiency

The perception of S starvation in plants occurs through complex genetic and metabolic mechanisms. During S deficiency, O-Acetylserine (OAS) begins to accumulate. Thus, the expression of genes encoding sulfate transporters and APS increases. The positive regulation of OAS has been suggested to be a signaling molecule in S starvation. GSH, on the contrary to OAS, plays a suppressive role in S assimilation [66].

Another response to S starvation is the suppression of GSL biosynthesis. The cell wants to regulate its energy and nutrient resources. Therefore, it suppresses the biosynthesis of the secondary metabolite GSL and directs its resources to primary metabolism, including cysteine and methionine. SLIM1 limits the activity of genes responsible for GSL biosynthesis, such as MYB28, by increasing the expression of the SDI1 and SDI2 genes [67] [68].

2.9 SLIM1 Transcription Factor

SLIM1 (S LIMITATION1) is a transcription factor belonging to the EIL (Ethylene-Insensitive3-Like) protein family. The EIL family includes proteins associated with the signaling pathway of ethylene, a hormone that is particularly effective in fruit ripening in plants. This protein family is activated by the ethylene hormone and initiates the relevant gene expression in the plant [69].

EIN3 is a transcription factor that plays a key role in ethylene signaling. It activates transcription by specifically binding to DNA sequences called PERE (Primary Ethylene Response Element). Other EIL proteins also function in the same signaling pathways as complements [70].Although SLIM1 is a member of the EIL protein family involved in ethylene signaling, it is a transcription factor that does not function in ethylene responses.

Studies have found that SLIM1 does not have the ability to bind to the PERE element, which is the basic DNA sequence that regulates the ethylene response. It has been stated that even if it binds to motifs related to ethylene signaling, it cannot interact strongly enough to initiate signaling because it can only bind temporarily [71]. Instead, unlike other EIL transcription factors, it functions in activating sulfate uptake, transporting sulfate, and regulating sulfate metabolism to maintain S homeostasis. It also controls sulfate utilization by suppressing the processes of glutathione and some secondary metabolites [72] [73].

In a study, it was observed that *slim1* mutants used could not induce the expression of the sulfate transporter SULTR1;2 under S deficiency conditions, and thus sulfate uptake decreased, which caused an approximately 60% decrease in sulfate uptake rate and a 30% shortening in root length. This shows the importance of the SLIM1 factor in S metabolism [74].

In addition, it is known that in *slim1* mutants, the expression level of SULTR1;1 is not reduced, on the contrary, its expression is induced. This shows that SLIM1 can act as an activator in case of S starvation and as a repressor in case of sufficient sulfate conditions. Sulfate deficiency, including *slim1* mutants, causes high expression of SULTR1;1. This shows that SLIM1 is critical for maintaining sulfate homeostasis in both low and normal conditions [75].

The transcription factor SLIM1 has been confirmed by many studies as a master regulator of sulfate metabolism. However, it has been discovered that there are other processes that SLIM1 acts on and has a function in regulating. Apart from sulfate starvation, it also acts to reduce plant responses to other stress conditions. Some of these are known to be active in plant defenses to reduce the harmful effects of elements that have toxicity, such as the metalloid arsenic and the heavy metal cadmium [76] [77].

On the other hand, SLIM1 also regulates the plant's sugar signaling. It controls glucose signals to maintain energy balance in the presence of S deficiency [78]. SLIM1 has also been shown to interact with jasmonate, ethylene and abscisic acid (ABA), and auxin. Jasmonate also indirectly increases the expression of genes that promote GSL biosynthesis, which in turn promotes the accumulation of indolic glucosinolates. By interacting with these hormones, SLIM1 helps the plant adapt to environmental stress [79].

There are conserved dimerization sequences in the n-terminal region of SLIM1. In this way, it binds to DNA as a homodimer. It is known that SLIM1 can bind to the TEBSS sequence to which it belongs. SLIM1 has been observed to bind to these sequences but has a very unstable interaction. Studies have shown that the UPE box, which contains two opposing TEBSS sequences, is a 20nt long binding region, especially seen in the promoters of genes related to sulfate starvation [80].

SLIM1 recognizes the UPE box motif through its N-terminal half region and homodimerization occurs. On the other hand, the C-terminal region of SLIM1 allows it to form heterodimers with EIN3, which belongs to the EIL protein family. This interaction between EIN3 and SLIM1 prevents SLIM1 from binding to DNA. Therefore, the dimer formation feature of SLIM1 significantly affects its ability to regulate sulfate metabolism [81].

In the first studies on sulfate starvation, SLIM1 was found to be associated with many genes and played an important role. In contrast, under S conditions, there was no change in the mRNA level of SLIM1, especially in shoots and roots. This suggested that SLIM1 is mainly regulated by post-transcriptional modifications. EIN3, which is from the same protein family as SLIM1, is known to be regulated by post-transcriptional modifications [82].

There are EBF1 and EBF2 proteins that recognize EIN3 and mark it with ubiquitin, thus responsible for its destruction under the necessary conditions. Studies have shown that these proteins interact with the C-terminal part of SLIM1. However, since the lysine parts responsible for its destruction remain outside this region, they do not cause its degradation [83].In addition, protein-protein interactions, another post-transcriptional modification, are also very effective in intracellular regulation. SLIM1 is known to interact with MYB75 and EIN3. These interactions help SLIM1 work more effectively in sulfate deficiency [84].

In a study, anthocyanin biosynthesis was investigated in *Arabidopsis thaliana* plants using two different genotypes, 35S::SLIM1 (SLIM1 overexpressing) and slim1-cr (CRISPR SLIM1 knockout). In 35S::SLIM1 plants, anthocyanin and flavonoid accumulation was observed, especially under S deficiency conditions. In slim1-cr, anthocyanin levels showed similar results in both S deficiency and S sufficient conditions. In addition, this study shows that SLIM1 increases anthocyanin accumulation by binding to the PAP1/MYB75 promoter with the Yeast 1 hybrid experiment [85].

Finally, sulfate metabolism is of vital importance for living things. Sulfate not only plays a role in the biosynthesis of amino acids such as methionine and cysteine, which contain S and are the basic building blocks of protein synthesis and metabolism, but also plays a critical role in the effectiveness of defense and antioxidant mechanisms.

In particular, compounds such as GSH, one of the most important products of sulfate metabolism, and glucosinolates and anthocyanins, which are secondary metabolites, play

important roles in plant defense. It increases the resistance of plants to environmental stresses. Recent studies have shown that sulfate metabolism also provides protection against the toxic effects of heavy metals and metalloids. Its effectiveness in B toxicity is particularly interesting. However, it is clear that not all roles of sulfate metabolism in defense mechanisms are yet fully understood and there are many areas to explore. Recent studies reveal some unknown pathways and potential regulatory elements of sulfate metabolism. These discoveries may make a great contribution to the development of plant species that are particularly resistant to environmental stresses.

The main aim of this project is to investigate the transcriptional profile of *slim1* mutants under different B toxicity in *Arabidopsis thaliana*. In particular, it is aimed to investigate the relationship between various metabolites and defense mechanisms in *slim1* mutant plants exposed to B toxicity. In the study, we will examine how plant metabolites such as glutathione, glucosinolates, phytochelatins and anthocyanins form a defense mechanism against B toxicity, and their connections with sulfate assimilation and antioxidant defense pathways. In addition, mechanisms that increase plant tolerance to B toxicity and sulfide deficiency will be examined. This study aims to understand how *A. thaliana* adapts under harsh environmental conditions such as B toxicity and sulfate starvation. It aims to provide new information on plant stress management and defense mechanisms.

3. MATERIALS & METHODS

3.1 Plant Materials, Growth and Treatment Conditions

In the study, wild type (WT) and *slim1* mutants *Arabidopsis thaliana* cv. Columbia seeds were used. In the first stage, *Arabidopsis thaliana* seeds were subjected to surface sterilization. In this stage, the seeds were inverted for 2 min in an Eppendorf tube containing 70% (v/v) EtOH, and then the EtOH was removed. Then, 2.5% (v/v) NaOCl was added, the seeds were inverted again for 10 min, and the NaOCl was removed.

After this stage, the seeds were washed three times with 500 µl of distilled water for 30 seconds. The surface-sterilized seeds were then planted in petri dishes. Control groups were grown in half-strength MS medium at a pH of 5.7. B toxicity treatments were also administered to *slim1* mutants [86].

For B toxicity treatment, the 1B treatment group was grown in medium containing 1 mM boric acid, while the 2B toxicity groups were grown in medium containing 2 mM boric acid. Petri dishes were wrapped in stretch film and aluminum foil. Seeds were subjected to cold stratification at 4°C for 3 days, then grown in a growth chamber at 22 ±1°C, with a photoperiod of 16 hours of light (200 µmol m–2 s–1) and 8 hours of darkness, maintaining 60% relative humidity for 14 days. All treatments were conducted with two replications.

3.2 RNA Isolation from *A. thaliana* Leaves

Arabidopsis thaliana leaves were ground using a pre-cooled mortar and pestle with liquid nitrogen and transferred to 1.5 ml pre-cooled Eppendorf tubes. 1 ml of TRIzol reagent was added to each sample and thoroughly mixed using a vortex mixer for 15 seconds. The mixture was centrifuged at 21,000g for 5 minutes at room temperature, and the supernatant was carefully collected. Approximately 900 µl of the supernatant was transferred to new tubes, and 180 µl of chloroform was added. After vortexing for 15 seconds, the samples were incubated at room temperature for 3 minutesand then centrifuged at 4°C, 21,000g for 15 minutes. The upper aqueous phase (around 400 µl) containing RNA was transferred to new tubes. An equal volume of isopropanolwas added, gently mixed, and incubated at room temperature for 10 minutes. Following incubation, the samples were centrifuged at 21,000g

for 10 minutes at room temperature, and the supernatant was discarded. The RNA pellet was washed with 75% ethanol, air-dried, and dissolved in RNase-free water.

RNA concentration and purity were measured using a NanoDrop spectrophotometer, and RNA integrity was verified via agarose gel electrophoresis.

3.3 RNA-Sequencing

The processing of the raw data obtained from the sequencing process was initiated in the Linux operating system and the expression levels of the genes were obtained in the iDEP 2.0 open access internet-based tool.

As the first step of the study, the raw data for data analysis was downloaded and quality control was performed. Both fastq files of the samples with short reads were obtained from the sequencer and the quality of the data was assessed using FastQC (v0.11.9). Then, the selected samples were processed using the cutadapt (v3.5) tool to remove adapter residues. After this process, quality control was performed again. The obtained results were summarized using MultiQC (v1.21) to investigate the variation before the alignment.

RNA-seq data were aligned to the TAIR10.58 *Arabidopsis thaliana* genome sequence using the Ensembl dataset with the STAR (Splice-aware aligner) tool.To use the STAR (v2.7.11b) tool, FASTA and GTF formatted annotation files containing the genome sequence were taken from the database and a whole genome index was created.Since different gene expressions were to be examined, 2-pass mode was opened in STAR and the obtained BAM formatted outputs were sorted. Then, the number of reads taken from a transcript was obtained with the --quantMode parameter and the read amounts of the transcripts were obtained.

The first two columns of the 4-column file containing the counts were used in the next analysis stage. The counts obtained from separate files for each sample to be used were combined. The resulting metadata files were prepared for gene analysis. The continuation of the study was carried out in iDEP.This web tool, DESeq2, which is an R/Bioconductor package, was used to understand the differences between the groups at the gene level; comparison was made using the DESeq2 count matrix and metadata files.

The size factor was estimated according to the distribution of each gene according to GLM (Gemeralized Linear Model) and differential expression was determined by the Wald test.

To increase statistical power, genes that were underexpressed before normalization were excluded from the study.Statistical significance of the samples was evaluated and listed according to p-value (<0.05) and log2 (Ifold change I>1).Transcripts classified as significant were visualized using the same web tool.For functional analysis of statistically significant transcripts, GSEA (Gene Set Enrichment Analysis) package was used to analyze Molecular Function, Biological Process and Cellular component in GeneOntology with KEGG datasets for their effects in biological context.

3.4 GO And KEGG Enrichment Analysis of Differentially Expressed Genes

Functional enrichment analysis of significant genes obtained from differential gene expression analysis was performed using ShinyGO (v0.77) web-based tool. In this analysis, differentially expressed genes (DEGs) were aligned to Gene Ontology (GO) and KEGG pathway enrichment databases. GO analysis allowed the identification of significant terms in molecular function and biological process categories. KEGG enrichment analysis was used to reveal significant changes in metabolic and signal transduction pathways.

ShinyGO performed a statistical analysis to associate gene sets with related pathways and ontologies. The significance of enriched terms was evaluated using thresholds of adjusted p value (Q value) ≤ 0.05. The analysis results were tabulated to show how biological processes and metabolic pathways are associated with differential gene expression.

3.5 Identification and Classification of Transcription Factors (TFs)

Differentially expressed transcription factor encoding genes were identified using the Plant Transcription Factor Database (PlantTFDB). To identify overlapping and condition-specific transcription factors expressed in different experimental conditions, analysis was performed using the DRAW Venn Diagram tool. Common transcription factors and condition-specific ones were identified as a result of this analysis.

4. RESULTS

4.1 Principal Component Analysis of Gene Expression in *slim1* Mutants and WT Under B Exposure

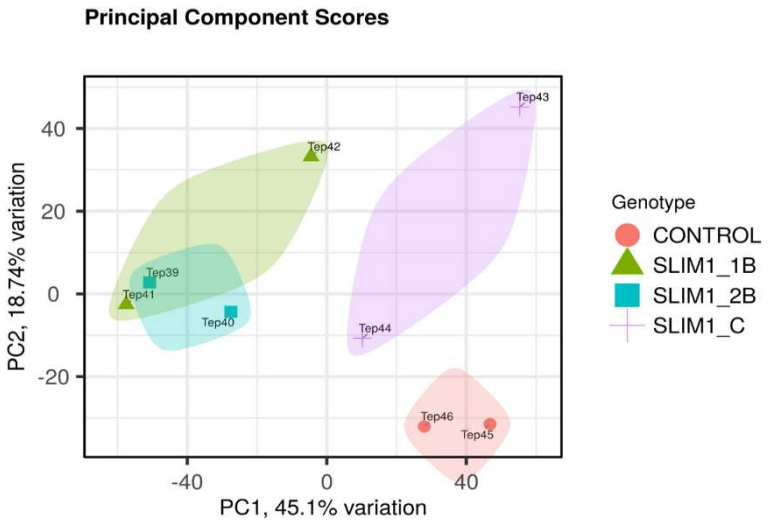

Figure 4. 1. Principal component analysis (PCA) of col-0, slim 1, and b-exposed slim 1_1B and slim1_2B mutants

Principal component analysis (PCA) of Arabidopsis *slim1* mutants was performed to examine the variation among different genotypes under various B treatments. PCA biplot shows the separation of genotypes based on the first two principal components (PC1 and PC2), which explain 63.84% of the total variation. The first principal component (PC1) explains 45.1% of the variation and the second principal component (PC2) explains 18.74%. Each genotype forms distinct clusters showing significant variation among groups.

In the graph, it is seen that each genotype is clustered in a different region. The control group is located in the lower right corner and shows a consistent distribution within itself. This shows that the control group, which was not exposed to B toxicity, has a more homogeneous biological response compared to the other genotypes and is more genotypically stable. It is clustered significantly differently from the control group and the *slim1* mutants.

The *slim1* mutant in condition 1B is clustered in the upper right. It was zoned differently from the other groups. The fact that the PC1 and PC2 values of *slim1* mutants in the 1B condition were located at points far from each other suggests that this species gives a different biological response to B toxicity compared to other genotypes. In other words, it

can be thought that *slim1* mutants in the 1B condition regulate genetic or biochemical processes at least very differently from the other groups against B toxicity.

The *slim1* mutant in condition 2B is clustered in the lower left corner and is located in a region that is quite separated from condition 1B and the control group. *Slim1* mutant in condition 2B appears to exhibit a more compact distribution within itself. This may indicate that the mutant gives a more homogeneous biological response to B toxicity. It can be suggested condition 2B gives a more stable response compared to the other groups and that its response to B toxicity is more consistent.

Finally, the *slim1* mutant control shows a separate clustering from the other groups in the upper right region. The PC1 and PC2 values of *slim1* mutant control are also located at distant points. It could not achieve a compact clustering. This may suggest that it has developed a different adaptive response to B toxicity. Although the *slim1* mutant control group had a similar structure to the *slim1* mutant in condition 1B, it may have developed a different response mechanism within itself.

PCA plot shows a separation between *slim1* mutants exposed to B toxicity and the control group. These different clusters may indicate that different biochemical pathways and adaptation strategies are triggered in each *slim1* mutant exposed to B toxicity. The control group did not show a uniform response to B toxicity and therefore showed a more stable and uniform clustering. Furthermore, *slim1* mutants in control and 1B condition were located on the positive side of PC1, whereas groups in 1B and 2B condition were located on the negative side of PC1, and PC1 captured most of the differences between genotypes. In general, the clusters were clearly separated from each other. This shows that the data we used in the analysis was correctly classified. A total of 63.84% of the variance data set was obtained from the first two components of the analysis. This provides a sufficient representation to understand the differences between the genotypes.

4.2 Raw Read Counts of *slim1* Mutants Under B Exposure

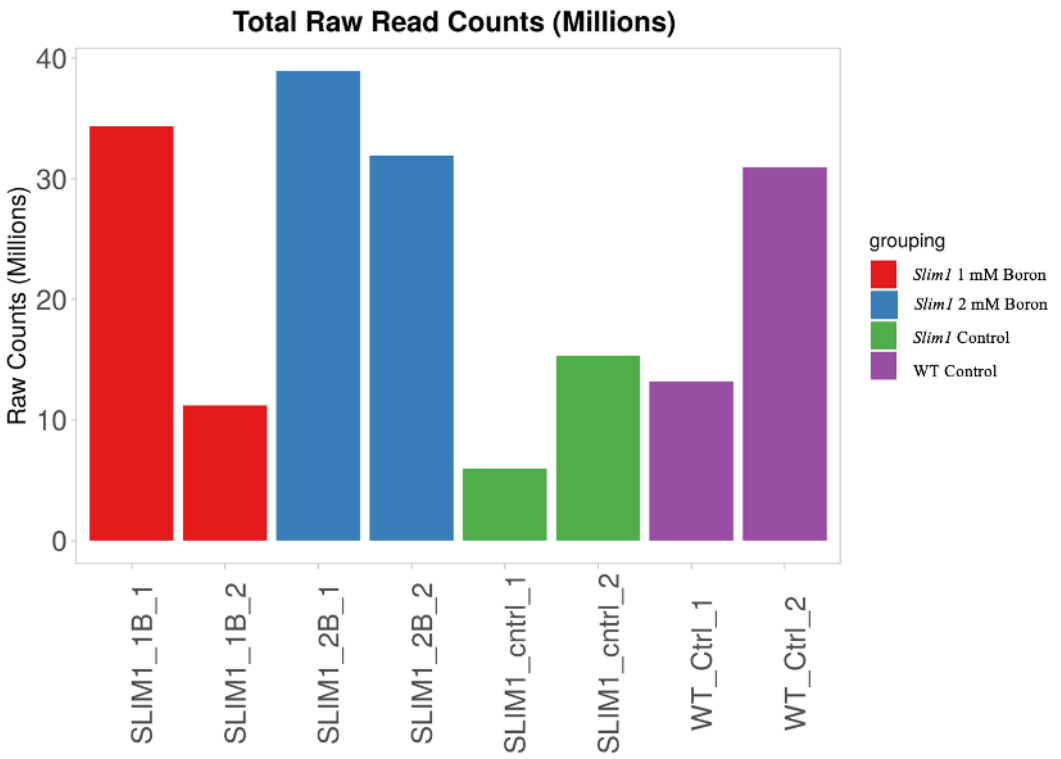

Figure 4. 2. Total raw read counts across *slim1* mutants and col-0 (WT) controls under different B conditions

The Raw Read Counts Graph shows the total raw read counts obtained for each sample, which vary between samples. These data help us understand the scope and depth of the RNA-Seq experiment. Each sample contains a sufficient number of reads to produce reliable and reproducible results for gene expression analyses. These results indicate that the data are sufficient in terms of reliability and depth. Additionally, there is sufficient data coverage for analyzing differences between genotypes and conditions.

Under the *slim1* mutant 2B condition, the raw read counts in the first replica and the second replica varied between 30-40 million, and this condition had the highest read depth. Especially, the first replica showed the highest read depth. In contrast, under the *slim1* 1B condition, the first replica had sufficient read counts, while the second replica gave a lower read count.

Replicas in the wild type (WT) condition have moderate raw read counts. Replicas in the *slim1* mutant control group have lower read depth compared to the other groups but still provide sufficient read counts for analysis. Overall, all samples have sufficient read depth, increasing the reliability of the dataset. However, the second replica under the *slim1* 1B condition may require more careful consideration in analysis due to low raw read counts.

4.3 Global Density Distribution of Transformed RNA-Seq Data

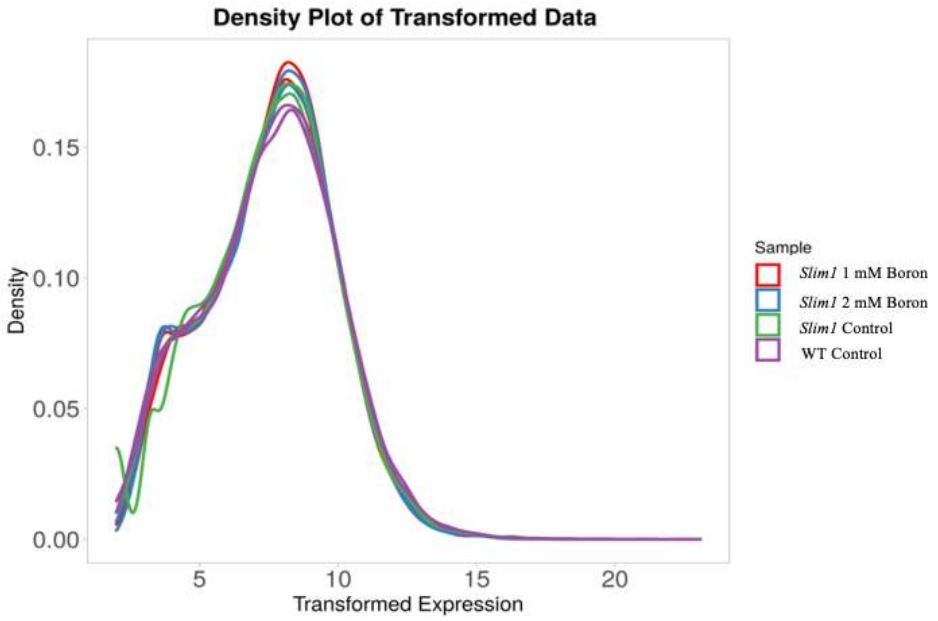

Figure 4. 3. Density plot of transformed gene expression data for *slim1* mutants and col-0 (WT) controls under B conditions

The Density Plot shows the density distributions of gene expression data obtained under different conditions. The curves in the plot have similar shapes and are located close to each other. This indicates that the data sets have generally similar distributions and that the normalization process has been successfully performed.

The peaks of the density curves in the same region indicate that the general gene expression profiles of the B-exposed group and the control group are significantly similar. However, the general distribution of the curves is similar. The slight deviations of the curves for each group from the other suggest that there may be small changes in gene expression levels between the groups.

The long tail on the right side of the plot indicates that the expression levels of some genes are higher than the others and that there is some asymmetry in the data. Although rare, the significantly increased expression levels of some genes suggest that B application or other experimental conditions may affect the genes at different levels. In general, this plot reveals that the groups do not show major differences in gene expression distributions, only small variations may occur.

4.4 Differential Gene Expression Between WT and *slim1* Under B Exposure

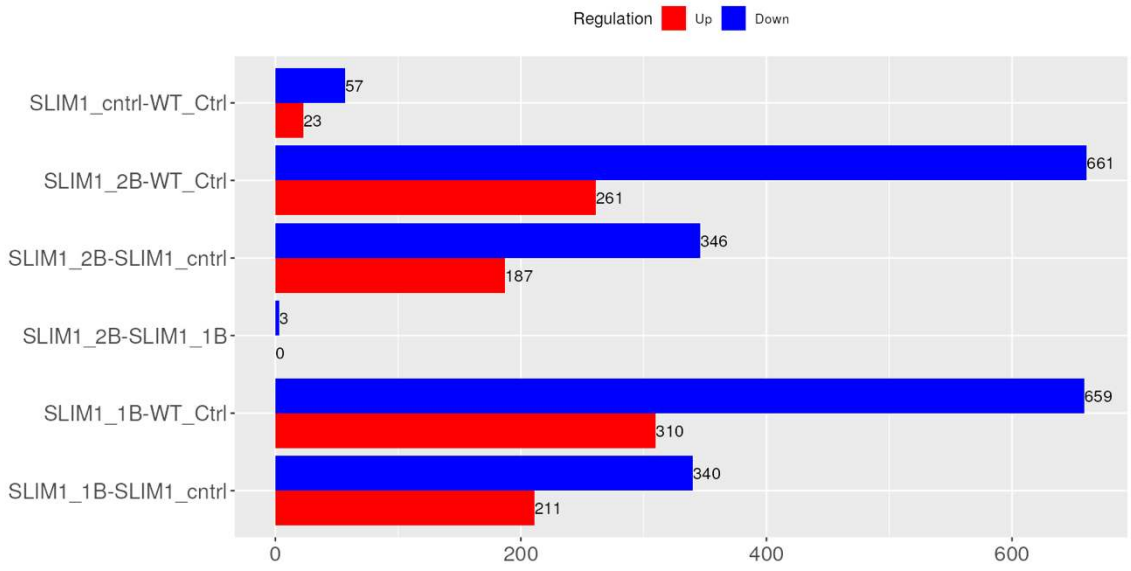

Figure 4. 4. Differential gene expression analysis in *slim1* mutants and col-0 (WT) plants under B toxicity conditions

The number of genes exhibiting up-regulation and down-regulation in gene expression levels of *slim1* mutants, compared to the WT control group, as well as *slim1* mutants exposed to B toxicity relative to the *slim1* control group, were analyzed. Additionally, the regulation levels of the *slim1* mutant under 1B and 2B conditions were also evaluated. In the comparison between *slim1* mutants in the 1B condition and the WT control

group, 310 genes were upregulated while 659 genes were down-regulated. In the comparison of the 2B condition with the WT control group, 261 genes showed upregulation and 661 genes demonstrated down-regulation. In the context of the comparison of the *slim1* mutant in the control condition, 211 genes were up-regulated, and 340 genes were down-regulated in the 1B condition. Similarly, under the 2B condition, 187 genes were upregulated and 346 were down-regulated. When directly comparing the 1B and 2B conditions, only 3 genes were down-regulated, and no upregulated genes were detected.

These findings suggest that *slim1* mutants have developed distinct condition-specific adaptation mechanisms in their transcriptional regulation against B toxicity. The high number of up- and down-regulated genes compared to the WT control group indicates the significant impact of B toxicity on gene expression in *slim1* mutants. Conversely, the minimal gene differences between 1B and 2B conditions suggest that similar molecular responses occur in both conditions

4.5 Venn Diagram of Genes Up- and Down-Regulated in *slim1* Mutants under B Toxicity

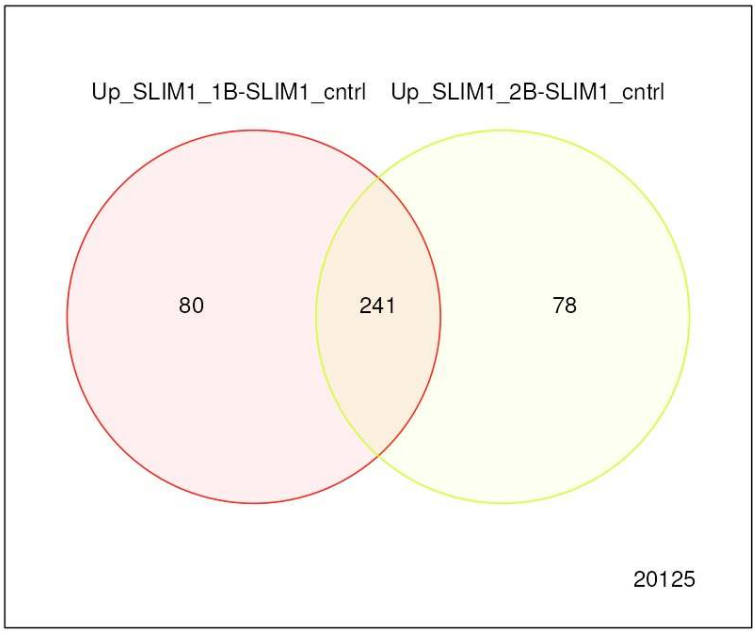

Figure 4. 5. Comparison of genes upregulated under 1B and 2B conditions of *slim1* mutants

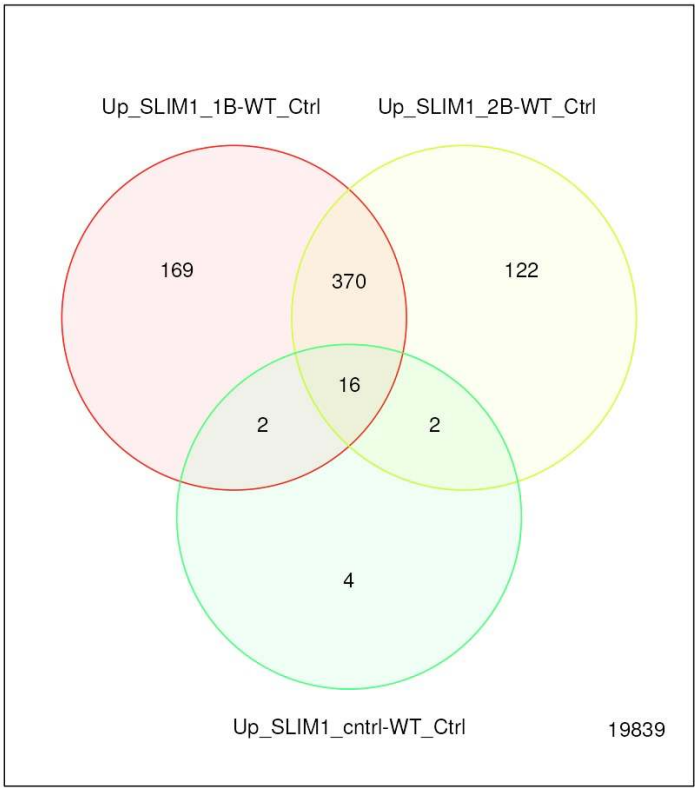

Figure 4. 6. Comparison of genes upregulated in *slim1* mutants compared to WT under 1B, 2B and control conditions

The genetic responses of *slim1* mutants to B toxicity were evaluated by analyzing up-regulated genes and the results were visualized with Venn diagrams. The first Venn diagram shows the similarities and differences between the genetic responses of *slim1* mutants under 1B and 2B conditions to B toxicity. There are 241 genes in common between *slim1* mutants under 1B and 2B conditions. These genes indicate the basic adaptation mechanisms developed by both mutants under toxic conditions. There are 80 genes specific to slim1 under 1B toxicity and 78 genes specific to *slim*1 under 2B toxicity.

The second Venn diagram compares the up-regulated genes developed by wt and *slim1* mutants to B toxicity. *slim1* mutants under 1B and 2B conditions share 370 genes, indicating basic genetic regulations developed against B toxicity. The fact that only 16 genes are common across all conditions indicates that a general adaptation to B toxicity is provided by a limited number of basic processes. The 169 genes specific to *slim1* 1b indicate that this mutant has developed a broad adaptation strategy. The 122 genes specific to *slim1* under 2B indicate adaptation mechanisms that develop a specific response. The wt group shares only 2-4 genes with *slim1* mutants, indicating a significant divergence in their genetic profiles due to the wt not being exposed to B toxicity.

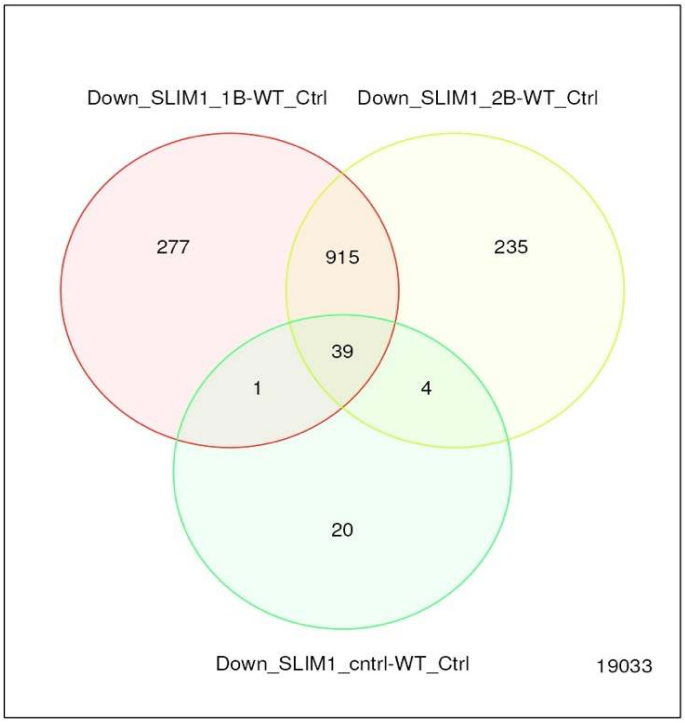

Figure 4. 7. Comparison of genes downregulated in *slim1* mutants compared to WT under 1B, 2B and control conditions

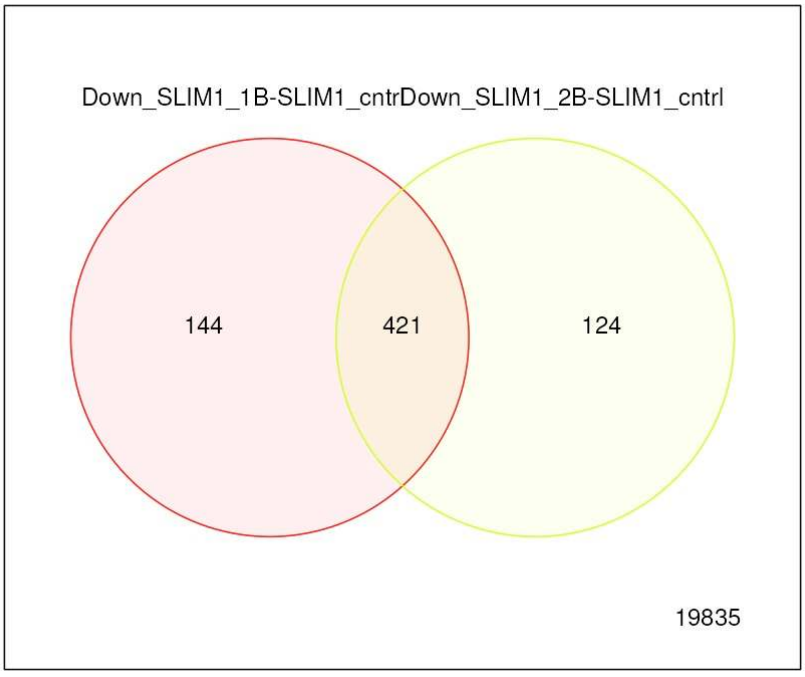

Figure 4. 8. Comparison of genes downregulated in *slim1* mutants under 1B and 2B conditions compared to controls

The first Venn diagram shows the similarities and differences between the genetic responses of *slim*1 1B, *slim1* 2B and *slim1* control groups to B toxicity. 915 common genes were downregulated in *sslim1* mutants under 1B and 2B conditions.These common genes reflect the basic adaptation mechanisms developed by both mutants against B toxicity. 277 genes specific to the *slim1* under 1B conditions group were downregulated. 235 genes specific to the *slim1* under 2B conditions group were downregulated. Only 20 genes were downregulated in the *slim1* control group. Only 39 genes were downregulated in all groups.

The second Venn diagram shows the genes downregulated in the *slim1* mutants under 1B and 2B conditions groups compared to the *slim1*control group. A total of 421 genes were observed to be downregulated in the *slim1* mutants under 1B and 2B conditions groups. In *slim1* 1b group, 144 genes and in *slim1* under 2B conditions, 124 genes were down-regulated only in these groups. This suggests that both groups developed different adaptation strategies against B toxicity. These analyses reveal genotype-specific differences in the adaptation of *slim1* mutants to B toxicity as well as common regulation of certain genetic processes.

4.6 Gene Ontology and KEGG Analyses

Table 4. 1. Significantly enriched GO biological processes for upregulated and downregulated genes in *slim1* control condition

Grup	Adj.Pval	Fold	GO ID	Pathway
Upregulated	7.07E-9	549.9	GO:0010438	Cellular response to sulfur starvation
	2.55E-5	33.9	GO:0009267	Cellular response to starvation
	2.55E-5	30.8	GO:0031669	Cellular response to nutrient levels
	2.55E-5	206.2	GO:1902358	Sulfate transmembrane transport
	3.18E-5	169.8	GO:0008272	Sulfate transport
	3.18E-5	27.2	GO:0042594	Response to starvation
	3.60E-5	15.5	GO:0006790	Sulfur compound metabolic process
	3.60E-5	25.2	GO:0031668	Cellular response to extracellular stimulus
	3.60E-5	24.4	GO:0071496	Cellular response to external stimulus
	3.95E-5	23.5	GO:0031667	Response to nutrient levels
Downregulated	6.25E-14	63.6	GO:0016144	S-glycoside biosynthetic process
	6.25E-14	63.6	GO:0019758	Glycosinolate biosynthetic process
	6.25E-14	63.6	GO:0019761	Glucosinolate biosynthetic process
	1.71E-12	22.1	GO:0044550	Secondary metabolite biosynthetic process
	1.71E-12	45.3	GO:1901659	Glycosyl compound biosynthetic process
	1.79E-12	13.5	GO:0019748	Secondary metabolic process
	1.79E-12	21.6	GO:0044272	Sulfur compound biosynthetic process
	5.28E-12	29.1	GO:0016143	S-glycoside metabolic process
	5.28E-12	29.1	GO:0019757	Glycosinolate metabolic process
	5.28E-12	29.1	GO:0019760	Glucosinolate metabolic process

Gene Ontology (GO) analysis was performed to evaluate the functional classification of genes based on the findings from differential gene expression analysis under *slim1* control conditions. This analysis provided insights into the roles of both up-regulated and down-regulated genes in biological processes.

Among the up-regulated genes, processes related to sulfur metabolism and the starvation response were notably prominent. The most enriched biological process was identified as "cellular response to sulfur starvation" (GO:0010438) with a 549.9-fold enrichment. Additionally, processes such as "cellular response to starvation" (GO:0009267),

"cellular response to nutrient levels" (GO:0031669), and "sulfate transport" (GO:0008272) are also significant.

In down-regulated genes, processes related to glucosinolate biosynthesis and its derivatives were found to be suppressed. For instance, a significant decrease was observed in processes such as the "S-glucoside biosynthetic process" (GO:0016144), "glucosinolate biosynthetic process" (GO:0019761), and "secondary metabolite biosynthetic process" (GO:0044550). These processes demonstrated up to 63.6-fold enrichment and were especially linked to the regulation of glucosinolate metabolism. Furthermore, pathways like the "sulfur compound biosynthetic process" (GO:0044272) were also suppressed.

Table 4. 2. Significantly enriched GO molecular functions for upregulated and downregulated genes in *slim1* control condition

Grup	Adj.Pval	Fold	GO ID	Pathway
Upregulated	4.30E-6	317	GO:0008271	Secondary active sulfate transmembrane transporter activity
	4.30E-6	271.7	GO:0015116	Sulfate transmembrane transporter activity
	4.81E-6	211.3	GO:0015301	Anion:anion antiporter activity
	4.81E-6	211.3	GO:0140323	Solute:anion antiporter activity
	2.11E-5	122.7	GO:1901682	Sulfur compound transmembrane transporter activity
	3.64E-4	45.3	GO:0015293	Symporter activity
	4.80E-4	39.2	GO:0015103	Inorganic anion transmembrane transporter activity
	1.97E-3	22.4	GO:0015297	Antiporter activity
	1.97E-3	23.2	GO:0022853	Active ion transmembrane transporter activity
	2.54E-3	19.8	GO:0008509	Anion transmembrane transporter activity
Downregulated	9.97E-3	124.5	GO:0003861	3-isopropylmalate dehydratase activity
	9.97E-3	166	GO:0047364	Desulfoglucosinolate sulfotransferase activity
	7.84E-2	62.3	GO:0000257	Nitrilase activity
	7.84E-2	62.3	GO:0003862	3-isopropylmalate dehydrogenase activity
	7.84E-2	83	GO:0003871	5-methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase activity
	7.84E-2	62.3	GO:0003958	NADPH-hemoprotein reductase activity
	7.84E-2	4.4	GO:0004497	Monooxygenase activity
	7.84E-2	124.5	GO:0004657	Proline dehydrogenase activity
	7.84E-2	33.2	GO:0008061	Chitin binding
	7.84E-2	38.3	GO:0008146	Sulfotransferase activity

Gene Ontology (GO) analysis was performed to profile the molecular functions of genes that were differentially up- and down-regulated in the *slim1* control condition.

When the up-regulated molecular functions, it was observed that sulfur transport activities were particularly prominent. There was a 317-fold enrichment in "secondary active sulfate transmembrane transporter activity" (GO:0008271) and a 271.7-fold enrichment in "sulfate transmembrane transporter activity" (GO:0015116), indicating that sulfur metabolism is significantly active in the *slim1* control condition. Additionally, there was a 211.3-fold enrichment in "anion:anion antiporter activity" (GO:0015301) and a 19.8-fold enrichment in "anion transmembrane transporter activity" (GO:0008509), highlighting an increase in the transport of ions, especially sulfate compounds, at the cellular level.

Among the down-regulated molecular functions, a suppression of glucosinolate metabolism and related enzymatic activities was observed. The “desulfoglucosinolate sulfotransferase activity” (GO:0047364), showing 166-fold enrichment, and the “5-methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase activity” (GO:0003871), exhibiting 83-fold enrichment, indicate a decrease in glucosinolate biosynthesis. Additionally, the “proline dehydrogenase activity” (GO:0004657) with 124.5-fold enrichment and “chitin binding” (GO:0008061) with 33.2-fold enrichment were also down-regulated. This indicates that metabolic priorities within the cell have shifted away from secondary metabolite processes like glucosinolate biosynthesis, and different metabolic pathways have emerged dominant.

Table 4. 3. Significantly enriched pathways for downregulated genes according to KEGG analysis in *slim1* control condition

Grup	Adj.Pval	Fold	Patway ID	Pathway
Downregulated	1.77E-12	65.8	Path:ath00966	Glucosinolate biosynthesis
	6.13E-7	18.6	Path:ath01210	2-Oxocarboxylic acid metabolism
	1.10E-3	28.8	Path:ath00290	Valine leucine and isoleucine biosynthesis
	7.12E-3	2.2	Path:ath01110	Biosynthesis of secondary metabolites

KEGG analysis was conducted under the *slim1* control condition. This analysis revealed pathways where significantly down-regulated genes were enriched. The metabolic processes involving these genes were characterized.

The analysis results indicated that the highest enrichment occurred in the "Glucosinolate biosynthesis" (Path:ath00966) pathway, with a 65.8-fold increase. This indicates that genes associated with glucosinolate biosynthesis were suppressed in the *slim1* control condition.

Additionally, "2-Oxocarboxylic acid metabolism" (Path:ath01210) exhibited an 18.6-fold enrichment, and "Valine, leucine and isoleucine biosynthesis" (Path:ath00290) showed a 28.8-fold enrichment, both of which are significant in these pathways. Furthermore, "Secondary metabolites biosynthesis" (Path:ath01110) was observed with a 2.2-fold enrichment.

Table 4. 4. Significantly enriched GO biological processes for upregulated and downregulated genes in *slim1* mutants under 1B condition

Grup	Adj.Pval	Fold	GO ID	Pathway
Upregulated	2.77E-9	3.7	GO:0042254	Ribosome biogenesis
	7.63E-8	3.1	GO:0022613	Ribonucleoprotein complex biogenesis
	3.38E-5	3.4	GO:0006364	rRNA processing
	3.87E-5	3.3	GO:0016072	rRNA metabolic process
	4.28E-4	6.2	GO:0000959	Mitochondrial RNA metabolic process
	2.66E-3	1.4	GO:0010467	Gene expression
	7.81E-3	2.3	GO:0034470	ncRNA processing
	7.81E-3	5.4	GO:0140053	Mitochondrial gene expression
	8.25E-3	7.1	GO:0000462	Maturation of SSU-rRNA from tricistronic rRNA transcript (SSU-rRNA 5.8S rRNA LSU-rRNA)
	1.09E-2	4.2	GO:0042273	Ribosomal large subunit biogenesis
Downregulated	1.76E-38	2.2	GO:0009628	Response to abiotic stimulus
	1.25E-35	1.9	GO:0042221	Response to chemical
	1.37E-32	1.7	GO:0006950	Response to stress
	2.23E-28	4.4	GO:0015979	Photosynthesis
	2.62E-25	2.9	GO:0009266	Response to temperature stimulus
	2.42E-23	2.1	GO:1901700	Response to oxygen-containing compound
	1.24E-22	5.4	GO:0019684	Photosynthesis light reaction
	1.44E-19	1.9	GO:0070887	Cellular response to chemical stimulus
	8.18E-18	5.7	GO:0016143	S-glycoside metabolic process
	8.18E-18	5.7	GO:0019757	Glycosinolate metabolic process

This Gene Ontology (GO) biological process analysis based on differential gene expression analysis of *slim1* mutants under the 1B condition shows the cellular functions of up-regulated and down-regulated genes.

The upregulated genes are primarily associated with ribosome biogenesis and ribonucleoprotein complex biogenesis. “Ribosome biogenesis” (GO:0042254) shows 3.7-fold enrichment, while “ribonucleoprotein complex biogenesis” (GO:0022613) displays 3.1-fold enrichment, suggesting that these processes contribute to increased translational activity and ribosome production. In addition, "rRNA processing” (GO:0006364) exhibits 3.4-fold enrichment, and “rRNA metabolic process” (GO:001607) demonstrates 3.3-fold enrichment, highlighting a focus on the biosynthesis of ribosomal RNA.

Pathways associated with mitochondrial RNA metabolism and gene expression were also upregulated. The 6.2-fold enrichment of the “mitochondrial RNA metabolic process” (GO:0000959) and the 5.4-fold enrichment of “mitochondrial gene expression” (GO:0140053) highlight the crucial role of mitochondria in cellular metabolism. These findings suggest that *slim1* mutants exhibit enhanced ribosomal and mitochondrial activities under the 1B condition.

The downregulated genes primarily relate to responses to stress and environmental stimuli. The 2.2-fold enrichment of “response to abiotic stimuli” (GO:0009628), the 1.9-fold enrichment of “chemical response” (GO:0042221), and the 1.7-fold enrichment of “response to stress” (GO:0006950) suggest that these genes are involved in responding to environmental stressors.

Pathways associated with photosynthesis were also suppressed. “Photosynthesis” (GO:0015979) exhibited 4.4-fold enrichment, and “light reaction” (GO:0019684) showed 5.4-fold enrichment, indicating reduced photosynthetic activity. Furthermore, “response to oxygen-containing compounds” (GO:1901700) demonstrated 2.1-fold enrichment, and “glucosinolate metabolic process” (GO:0019757) exhibited 5.7-fold enrichment, both of which were down-regulated.

Table 4. 5. Significantly enriched GO molecular functions for upregulated and downregulated genes in *slim1* mutants under 1B condition

Grup	Adj.Pval	Fold	GO ID	Pathway
Upregulated	6.91E-4	11.8	GO:0030515	snoRNA binding
	1.65E-3	2.5	GO:0140098	Catalytic activity acting on RNA
	1.91E-3	1.4	GO:0003676	Nucleic acid binding
	6.33E-3	2.4	GO:0003735	Structural constituent of ribosome
	8.62E-3	1.6	GO:0003723	RNA binding
	8.63E-3	4.3	GO:0003724	RNA helicase activity
	8.63E-3	4.3	GO:0008186	ATP-dependent activity acting on RNA
	3.60E-2	1.9	GO:0140640	Catalytic activity acting on a nucleic acid
	4.87E-2	5.4	GO:0003899	DNA-directed 5-prime-3-prime RNA polymerase activity
	5.32E-2	8.2	GO:0015301	Anion:anion antiporter activity
Downregulated	8.40E-11	5.9	GO:0019904	Protein domain specific binding
	1.97E-9	1.8	GO:0016491	Oxidoreductase activity
	2.56E-9	9.3	GO:0005372	Water transmembrane transporter activity
	2.56E-9	9.3	GO:0015250	Water channel activity
	7.31E-9	7.4	GO:0016168	Chlorophyll binding
	4.79E-7	7.4	GO:0016762	Xyloglucan:xyloglucosyl transferase activity
	1.98E-5	3.4	GO:0051082	Unfolded protein binding
	8.51E-5	2.2	GO:0046906	Tetrapyrrole binding
	6.28E-4	3.4	GO:0030247	Polysaccharide binding
	8.05E-4	5.3	GO:0005200	Structural constituent of cytoskeleton

Gene Ontology (GO) analysis of the molecular functions of genes under the 1B condition in *slim1* mutants reveals the biological roles of both up- and down-regulated genes.

The up-regulated genes are prominent in functions related to RNA processing and ribosome structure. The 11.8-fold enrichment of “snoRNA binding” (GO:0030515) and the 2.5-fold enrichment of “catalytic activity acting on RNA” (GO:0140098) indicate that RNA processing activities are significantly active. Additionally, the 2.4-fold enrichment of “structural component of ribosome” (GO:0003735) and the 4.3-fold enrichment of “RNA

helicase activity" (GO:0003724) indicate an increase in the structural and functional configurations of ribosomes and RNA for protein synthesis.

These pathways also demonstrate an increase in transcriptional processes, showing a 4.3-fold enrichment in "ATP-dependent RNA-acting activity" (GO:0008186) and a 5.4-fold enrichment in "DNA-directed RNA polymerase activity" (GO:0003899). This indicates an increased focus on RNA synthesis and processing under the 1B condition of *slim1* mutants.

The down-regulated genes are involved in processes such as water transport, photosynthesis-related pigment binding, and protein regulation. A 9.3-fold enrichment in "Water transmembrane transporter activity" (GO:0005372) and a 7.4-fold enrichment in "chlorophyll binding" (GO:0016168) indicate suppression of photosynthetic activities and a decrease in water transport. In addition, the 5.9-fold enrichment of "protein domain specific binding" (GO:0019904) and the 3.4-fold enrichment of "unfolded protein binding" (GO:0051082) indicate suppression of protein regulation and quality control mechanisms.

The 3.4-fold enrichment of "structural polysaccharide binding" (GO:0030247) and the 5.3-fold enrichment of "structural component of cytoplasm skeleton" (GO:0005200) reflect the decrease in these pathways, cell wall and cytoskeletal processes. In addition, the 1.8-fold enrichment of "oxidoreductase activity" (GO:0016491) and the 2.2-fold enrichment of "tetrapyrrole binding" (GO:0046906) indicate changes in oxidative metabolism and pigment metabolism.

Table 4. 6. Significantly enriched pathways for upregulated and downregulated genes according to KEGG analysis in *slim1* mutants under 1B condition

Grup	Adj.Pval	Fold	Pathway ID	Pathway
Upregulated	2.10E-5	6.2	Path:ath03008	Ribosome biogenesis in eukaryotes
Downregulated	2.64E-16	1.5	Path:ath01100	Metabolic pathways
	3.35E-14	7.1	Path:ath00195	Photosynthesis
	3.35E-14	10	Path:ath00966	Glucosinolate biosynthesis
	2.32E-9	8.4	Path:ath00196	Photosynthesis-antenna proteins
	9.75E-9	1.6	Path:ath01110	Biosynthesis of secondary metabolites
	3.60E-5	3.4	Path:ath01210	2-Oxocarboxylic acid metabolism
	1.53E-3	3.7	Path:ath00920	Sulfur metabolism
	1.55E-3	3.6	Path:ath00592	Alpha-Linolenic acid metabolism
	1.55E-3	2.9	Path:ath00710	Carbon fixation in photosynthetic organisms
	1.25E-2	2.8	Path:ath00860	Porphyrin metabolism

KEGG analysis based on differential gene expression of *slim1* mutants in the 1B condition shows which metabolic pathways the up- and down-regulated genes are involved in.The up-regulated genes are particularly associated with ribosome biogenesis in eukaryotes. "Ribosomal biogenesis in eukaryotes" (Path:ath03008) with a 6.2-fold enrichment indicates an increase in the production of ribosomal components and that these genes support translational activity.

The down-regulated genes are generally associated with metabolic processes. The most notable suppressed pathways include "Photosynthesis" (Path:ath00195) with a 7.1-fold enrichment and "Photosynthesis-antenna proteins" (Path:ath00196) with an 8.4-fold enrichment. The suppression of these pathways suggests that photosynthetic activities are reduced in *slim1* mutants and that energy metabolism is shifted to a different direction.

“Glucosinolate biosynthesis” (Path:ath00966) was significantly suppressed with a 10-fold enrichment. In addition, “2-Oxocarboxylic acid metabolism” (Path:ath01210) was suppressed with a 3.4-fold enrichment, “Sulfur metabolism” (Path:ath00920) with a 3.7-fold enrichment, and “Secondary metabolites biosynthesis” (Path:ath01110) with a 1.6-fold enrichment. These findings indicate changes in the regulation of basic metabolic processes and processes related to sulfur metabolism.Other suppressed pathways include “Carbon fixation” (Path:ath00710) with a 2.9-fold enrichment, and “Porphyrin metabolism” (Path:ath00860) with a 2.8-fold enrichment.

Table 4. 7. Significantly enriched GO biological processes for upregulated and downregulated genes in *slim1* mutants under 2B condition

Grup	Adj.Pval	Fold	GO ID	Pathway
Upregulated	6.80E-7	3.4	GO:0042254	Ribosome biogenesis
	2.27E-6	3	GO:0022613	Ribonucleoprotein complex biogenesis
	6.38E-4	3.2	GO:0006364	rRNA processing
	6.88E-4	3.1	GO:0016072	rRNA metabolic process
	8.30E-3	5.7	GO:0140053	Mitochondrial gene expression
	1.05E-2	5.4	GO:0000959	Mitochondrial RNA metabolic process
	1.05E-2	1.4	GO:0010467	Gene expression
	1.45E-2	29.2	GO:0006390	Mitochondrial transcription
	3.12E-2	13	GO:0031126	sno(s)RNA 3-prime-end processing
	3.12E-2	2.2	GO:0034470	ncRNA processing
Downregulated	3.54E-31	2.1	GO:0009628	Response to abiotic stimulus
	6.55E-31	1.9	GO:0042221	Response to chemical
	7.12E-30	1.7	GO:0006950	Response to stress
	2.74E-21	2.8	GO:0009266	Response to temperature stimulus
	2.36E-18	1.9	GO:0070887	Cellular response to chemical stimulus
	2.39E-18	1.9	GO:1901700	Response to oxygen-containing compound
	1.74E-16	2.1	GO:0043207	Response to external biotic stimulus
	1.74E-16	2.1	GO:0051707	Response to other organism
	2.64E-16	3.7	GO:0071456	Cellular response to hypoxia
	2.79E-16	2	GO:0044419	Biological process involved in interspecies interaction between organisms

This Gene Ontology biological process analysis, based on the differential gene expression of *slim1* mutants under 2B conditions, demonstrates the cellular functions of both up- and down-regulated genes.

The up-regulated genes are primarily related to ribosome biogenesis and mitochondrial gene expression. “Ribosome biogenesis” (GO:0042254) is enriched 3.4-fold and “ribonucleoprotein complex biogenesis” (GO:0022613) is enriched 3-fold, indicating that these processes increase translational activity in *slim1* mutants. Furthermore, “rRNA processing” (GO:0006364) is enriched 3.2-fold and “rRNA metabolic process” (GO:0016072) is enriched 3.1-fold, indicating a focus on ribosomal RNA production.

Processes related to mitochondrial activity are also up-regulated. “Mitochondrial gene expression” (GO:0140053) is enriched 5.7-fold, “mitochondrial RNA metabolic process” (GO:0000959) is enriched 5.4-fold, and “mitochondrial transcription” (GO:0006390) is enriched 29.2-fold, indicating that mitochondrial functions are

significantly enhanced under these conditions. Furthermore, “snoRNA 3’ end processing” (GO:0031126) is enriched 13-fold, indicating an increase in RNA editing processes.

The down-regulated genes are involved in processes related to stress responses and adaptation to environmental stimuli. "Response to abiotic stimuli" (GO:0009628) with 2.1-fold enrichment, "chemical response" (GO:0042221) with 1.9-fold enrichment, and "response to stress" (GO:0006950) with 1.7-fold enrichment indicates that these processes are suppressed in response to environmental stress factors.

Additionally, "cellular response to hypoxia" (GO:0071456) shows 3.7-fold enrichment, and "response to oxygen-containing compounds" (GO:1901700) demonstrates 1.9-fold enrichment, indicating that these processes are reduced in response to oxidative stress. The "Response to external biotic stimuli" (GO:0043207) with 2.1-fold enrichment and the "response to other organisms" (GO:0051707) with the same enrichment level also exhibit suppression of biotic stress-related pathways. Furthermore, the suppression of these pathways with 2-fold enrichment in "biological processes associated with interspecies interactions" (GO:0044419) reveals that these mutants have reduced adaptive capacities to environmental and biotic factors.

Table 4. 8. Significantly enriched GO molecular functions for upregulated and downregulated genes in *slim1* mutants under 2B condition

Grup	Adj.Pval	Fold	GO ID	Pathway
Upregulated	2.52E-4	1.7	GO:0005524	ATP binding
	2.52E-4	1.6	GO:0035639	Purine ribonucleoside triphosphate binding
	3.23E-4	1.6	GO:0017076	Purine nucleotide binding
	3.23E-4	1.6	GO:0030554	Adenyl nucleotide binding
	3.23E-4	1.6	GO:0032555	Purine ribonucleotide binding
	3.23E-4	1.6	GO:0032559	Adenyl ribonucleotide binding
	3.55E-4	1.5	GO:0032553	Ribonucleotide binding
	5.41E-4	1.5	GO:0097367	Carbohydrate derivative binding
	1.91E-3	2.4	GO:0140098	Catalytic activity acting on RNA
	2.84E-3	4.7	GO:0003724	RNA helicase activity
Downregulated	4.05E-12	1.9	GO:0016491	Oxidoreductase activity
	1.75E-9	9.8	GO:0005372	Water transmembrane transporter activity
	1.75E-9	9.8	GO:0015250	Water channel activity
	3.93E-7	7.7	GO:0016762	Xyloglucan:xyloglucosyl transferase activity
	2.47E-6	3.7	GO:0051082	Unfolded protein binding
	3.12E-5	2.3	GO:0046906	Tetrapyrrole binding
	3.91E-5	4.2	GO:0019904	Protein domain specific binding
	1.89E-4	4.7	GO:0031072	Heat shock protein binding
	2.92E-4	2.2	GO:0016705	Oxidoreductase activity acting on paired donors with incorporation or reduction of molecular oxygen
	2.92E-4	3.6	GO:0030247	Polysaccharide binding

Gene Ontology (GO) analysis based on differential gene expression of *slim1* mutants under 2B condition shows molecular functions of up and down-regulated genes.

Energy metabolism and RNA processing processes are seen to be prominent in up-regulated molecular functions. The 1.7-fold enrichment of "ATP binding" (GO:0005524) and the 1.6-fold enrichment of "purine ribonucleoside triphosphate binding" (GO:0035639) indicate that energy production and nucleotide metabolism are active under this condition. However, the 4.7-fold enrichment of "RNA helicase activity" (GO:0003724) and the 2.4-fold enrichment of "RNA-effective catalytic activity" (GO:0140098) indicate an increase in these processes, RNA regulation, and transcriptional activities.

Among the down-regulated molecular functions, it is noteworthy that the pathways related to water transport, protein quality control, and oxidative metabolism are suppressed.

The suppression of these pathways with a 9.8-fold enrichment in "water transmembrane transporter activity" (GO:0005372) and a 9.8-fold enrichment in "water channel activity" (GO:0015250) indicates that the capacity of the cell to regulate water homeostasis is reduced. In addition, the weakening of these processes with a 3.7-fold enrichment in "unfolded protein binding" (GO:0051082, 3.7) and a 4.7-fold enrichment in "heat shock protein binding" (GO:0031072) indicates that defense mechanisms for protein stability are reduced.

However, a significant decrease in these processes was also observed with a 1.9-fold enrichment in "oxidoreductase activity" (GO:0016491) and a 7.7-fold enrichment in "xiloglucan:xiloglucosyl transferase activity" (GO:0016762).

Table 4. 9. Significantly enriched pathways for upregulated and downregulated genes according to KEGG analysis in *slim1* mutants under 2B condition

Grup	Adj.Pval	Fold	Pathway ID	Pathway
Upregulated	1.17E-3	5.1	Path:ath03008	Ribosome biogenesis in eukaryotes
	1.70E-2	5.7	Path:ath00280	Valine leucine and isoleucine degradation
	5.22E-2	3.3	Path:ath03018	RNA degradation
Downregulated	2.27E-14	10.8	Path:ath00966	Glucosinolate biosynthesis
	2.23E-12	7	Path:ath00195	Photosynthesis
	2.15E-11	1.4	Path:ath01100	Metabolic pathways
	5.50E-6	1.5	Path:ath01110	Biosynthesis of secondary metabolites
	2.99E-4	3.3	Path:ath01210	2-Oxocarboxylic acid metabolism
	5.48E-4	5.6	Path:ath00196	Photosynthesis-antenna proteins
	7.53E-4	2.1	Path:ath04141	Protein processing in endoplasmic reticulum
	5.12E-3	1.8	Path:ath04075	Plant hormone signal transduction
	1.22E-2	2.9	Path:ath00380	Tryptophan metabolism
	1.54E-2	3.2	Path:ath00592	Alpha-Linolenic acid metabolism

KEGG analysis of *slim1* mutants under 2B condition shows which metabolic processes the up- and down-regulated genes are involved in.

When the up-regulated pathways are examined, it is seen that especially the pathways related to protein synthesis and amino acid metabolism stand out. "Ribosomal biogenesis in eukaryotes" (Path:ath03008) showed 5.1-fold enrichment, indicating that the production of ribosomal components is increased and translational processes are supported. In addition,

"valine, leucine and isoleucine degradation" (Path:ath00280) showed 5.7-fold enrichment. In addition, the "RNA degradation" (Path:ath03018) pathway was enriched 3.3-fold, indicating that RNA editing and fragmentation processes contribute to energy conversion.

When the downregulated pathways are examined, it becomes apparent that crucial metabolic processes such as photosynthesis, glucosinolate biosynthesis, and hormone signaling are suppressed. Glucosinolate biosynthesis (Path:ath00966) is downregulated in this pathway with a 10.8-fold enrichment, indicating that defense mechanisms are weakened. Additionally, photosynthesis (Path:ath00195) shows a 7-fold enrichment, and photosynthesis-antenna proteins (Path:ath00196) exhibit a 5.6-fold enrichment, suggesting that energy production processes are hindered under this condition.

In addition, "protein processing" (Path:ath04141) is 2.1-fold enriched and "plant hormone signaling" (Path:ath04075) is 1.8-fold enriched, indicating that cellular regulation and adaptation mechanisms are suppressed.

Table 4. 10. Up-regulated transcription factors in *slim1* mutants under 1B condition

AGI ID	Gene Name	Log2 FC	P-Value
AT5G14490	ANAC085	5,11	3,39E-03
AT3G01600	ANAC044	4,54	6,22E-04
AT5G64060	ANAC103	3,23	2,84E-06
AT1G29860	WRKY71	2,93	2,93E-02
AT4G28790	BHLH23	2,70	1,05E-02
AT3G05690	UNE8	2,00	7,87E-08
AT5G22890	STOP2	1,81	6,29E-04
AT2G25000	WRKY60	1,67	1,66E-04
AT5G67450	AZF1	1,64	2,15E-03
AT4G24150	GRF8	1,63	3,30E-02
AT5G43290	WRKY49	1,61	2,28E-02
AT1G74650	ATY13	1,55	1,42E-03
AT5G15130	WRKY72	1,50	1,90E-02
AT1G30650	WRKY14	1,41	2,76E-03
AT3G44750	HDT1	1,39	1,48E-02
AT1G54160	NFYA5	1,36	4,13E-02
AT3G61250	ATMYB17	1,29	3,30E-02
AT1G75520	SRS5	1,27	2,16E-02
AT5G60910	AGL8	1,26	3,42E-02
AT2G02060		1,23	3,51E-02
AT1G77920	TGA7	1,20	1,43E-03
AT2G31280	CPUORF7	1,19	6,62E-06
AT2G36270	ABI5	1,09	6,81E-03
AT4G35900	FD	1,07	2,15E-03
AT1G46264	HSFB4	1,06	3,57E-02
AT1G28360	ERF12	1,02	2,19E-02
AT4G38620	MYB4	1,00	3,16E-02
AT5G15210	ZHD8	1,00	6,09E-03
AT5G60690	REV	0,95	3,92E-02
AT2G45050	GATA2	0,92	3,42E-02
AT3G22780	TSO1	0,87	4,54E-03
AT3G48440		0,85	2,82E-02
AT1G72830	HAP2C	0,82	4,15E-02
AT3G48430	REF6	0,82	2,15E-03
AT4G19990	FRS1	0,79	2,97E-02
AT4G39160		0,73	3,29E-02
AT3G26744	SCRM	0,70	1,49E-02
AT5G60450	ARF4	0,68	4,58E-02

Transcription factors that were up-regulated under the 1B condition in *slim1* mutants were identified based on gene expression analysis results. ANAC085 (Log2 FC: 5.11; P-Value: 3.39E-03), ANAC044 (Log2 FC: 4.54; P-Value: 6.22E-04), and ANAC103 (Log2 FC: 3.23; P-Value: 2.84E-06) represent some of the most highly up-regulated genes in the NAC family. Among the WRKY family factors, WRKY71 (Log2 FC: 2.93; P-Value: 2.93E-02), WRKY60 (Log2 FC: 1.67; P-Value: 1.66E-04), WRKY49 (Log2 FC: 1.61; P-Value: 2.28E-02), and WRKY14 (Log2 FC: 1.41; P-Value: 2.76E-03) were significantly up-regulated.

Additionally, transcription factors such as STOP2 (Log2 FC: 1.81; P-Value: 6.29E-04), BHLH23 (Log2 FC: 2.70; P-Value: 1.05E-02), HDT1 (Log2 FC: 1.39; P-Value: 1.48E-02), MYB4 (Log2 FC: 1.00; P-Value: 3.16E-02), and ABI5 (Log2 FC: 1.09; P-Value: 6.81E-03) were also up-regulated. Some of these factors are related to metabolism, stress response, and gene regulation. These results indicate that *slim1* mutants exhibit significant changes in transcriptional regulation under the 1B condition.

Table 4. 11. Down-regulated transcription factors in *slim1* mutants under 1B condition

AGI ID	Gene Name	log2 FC	P-value
AT2G26150	HSFA2	-5,45	4,89E-07
AT5G07700	MYB76	-4,05	3,54E-04
AT5G07690	MYB29	-3,52	1,94E-13
AT2G40750	WRKY54	-3,04	1,39E-11
AT5G39860	PRE1	-1,86	8,10E-03
AT4G06746	RAP2-9	-1,69	9,57E-05
AT1G73870	COL7	-1,69	2,45E-05
AT2G42380	BZIP34	-1,60	9,46E-08
AT2G44910	ATHB-4	-1,50	1,18E-02
AT3G58120	BZIP61	-1,47	5,09E-08
AT1G77640	ERF013	-1,45	5,31E-04
AT5G62430	CDF1	-1,41	3,56E-02
AT2G40340	DREB2C	-1,41	5,88E-05
AT5G17490	RGL3	-1,38	2,47E-03
AT3G06490	MYB108	-1,33	3,68E-02
AT1G12610	DREB1F	-1,25	4,88E-03
AT1G63030	DREB1E	-1,24	2,22E-02
AT4G16780	HAT4	-1,23	2,44E-04
AT4G17810		-1,22	3,89E-04
AT2G39250	SNZ	-1,20	1,31E-02
AT1G06040	BBX24	-1,19	1,27E-06
AT5G08130	BIM1	-1,19	3,50E-04
AT3G25710	BHLH32	-1,18	2,41E-03
AT1G26945	PRE6	-1,18	1,98E-03
AT4G34410	ERF109	-1,18	1,98E-02
AT5G62470	MYB96	-1,18	1,32E-02
AT1G21910	ERF012	-1,16	1,11E-03
AT1G33760	ERF022	-1,16	5,37E-03
AT2G43010	PIF4	-1,15	1,24E-05
AT5G57660	COL5	-1,12	2,75E-04
AT4G17490	ERF6	-1,11	9,44E-06
AT3G11020	DREB2B	-1,10	3,82E-02
AT5G44260	TZF5	-1,10	1,11E-03
AT1G33240	AT-GTL1	-1,06	4,03E-04
AT2G18300		-1,03	5,91E-04
AT4G32800	ERF043	-1,02	2,64E-02
AT3G50260	ERF011	-1,02	3,00E-03
AT3G47620	TCP14	-1,02	1,26E-04

AT1G18330	RVE7 AT1G18330	-1,01	2,56E-03
AT2G44840	ERF13	-0,99	1,20E-02
AT4G31800	WRKY18	-0,97	1,67E-02
AT2G02070	IDD5	-0,96	1,62E-04
AT3G06590	BHLH148	-0,96	2,28E-03
AT1G13260	RAV1	-0,95	1,74E-04
AT4G36540	BEE2	-0,92	7,51E-03
AT1G52880	NAC018	-0,92	3,38E-02
AT1G27730	ZAT10	-0,90	5,97E-04
AT3G56400	WRKY70	-0,90	1,37E-02
AT4G17230	SCL13	-0,84	2,93E-02
AT1G14580		-0,84	1,27E-02
AT5G61590	ERF107	-0,84	1,44E-02
AT5G05410	DREB2A	-0,84	6,95E-03
AT5G48250	COL10	-0,82	9,05E-03
AT2G38470	WRKY33	-0,81	2,18E-02
AT1G76890	GT-2	-0,80	7,88E-03
AT1G19350	BES1	-0,78	9,76E-03
AT5G67300	MYB44	-0,76	3,64E-02
AT5G52010		-0,76	4,53E-02
AT2G26580	YAB5	-0,75	4,33E-02
AT3G54810	GATA8	-0,75	1,70E-02
AT3G46640	PCL1	-0,75	3,65E-02
AT1G50640	ERF3	-0,73	2,82E-02
AT4G17460	HAT1	-0,73	2,63E-02
AT5G23280	TCP7	-0,67	4,76E-02
AT5G47370	HAT2	-0,67	1,07E-02
AT5G13180	NAC083	-0,63	3,53E-02
AT3G55980	SZF1	-0,59	4,33E-02

Differential gene expression analysis of *slim1* mutants under 1B conditions revealed that many genes from various transcription factor families were downregulated.

The NAC family genes NAC083 (Log2 FC: -0.63) and NAC018 (Log2 FC: -0.92) were suppressed. Genes from the MYB family, including MYB76 (Log2 FC: -4.05), MYB29 (Log2 FC: -3.52), MYB108 (Log2 FC: -1.33), MYB96 (Log2 FC: -1.18), and MYB44 (Log2 FC: -0.76), were also significantly downregulated.

Transcription factors belonging to the WRKY family, such as WRKY54 (Log2 FC: -3.04), WRKY70 (Log2 FC: -0.90), and WRKY33 (Log2 FC: -0.81), were similarly repressed. Additionally, the DREB family genes DREB2C (Log2 FC: -1.41), DREB1F

(Log2 FC: -1.25), DREB1E (Log2 FC: -1.24), and DREB2B (Log2 FC: -1.10) showed downregulation. Furthermore, genes belonging to the ERF family, including ERF013 (Log2 FC: -1.45), ERF6 (Log2 FC: -1.11), ERF012 (Log2 FC: -1.16), and ERF109 (Log2 FC: -1.18), were also repressed.

These results suggest that *slim1* mutants adopt a different regulatory strategy in managing transcription factors under 1B conditions, altering their effects on stress responses and developmental processes.

Table 4. 12. Up-regulated transcription factors in *slim1* mutants under 2B condition

AGI ID	Gene Name	log2 FC	P-value
AT5G14490	anac085	4,89	5,54E-03
AT3G01600	anac044	4,72	3,22E-04
AT5G64060	anac103	3,30	1,26E-06
AT4G28790	BHLH23	2,42	2,50E-02
AT3G05690	UNE8	1,80	1,45E-06
AT5G65070	MAF4	1,80	2,41E-02
AT4G24150	GRF8	1,69	2,17E-02
AT5G22890	STOP2	1,69	1,31E-03
AT1G74650	ATY13	1,55	1,07E-03
AT5G67450	AZF1	1,48	6,77E-03
AT1G30650	WRKY14	1,44	1,46E-03
AT3G44750	HDT1	1,38	1,49E-02
AT2G31280	CPUORF7	1,37	5,47E-08
AT2G25000	WRKY60	1,35	3,57E-03
AT1G77920	TGA7	1,34	1,89E-04
AT5G60690	REV	1,30	1,45E-03
AT5G60910	AGL8	1,27	2,84E-02
AT1G36060	ERF055	1,25	3,63E-03
AT2G02060		1,24	3,07E-02
AT1G19210	ERF017	1,10	3,73E-02
AT1G32870	ANAC13	1,00	1,43E-02
AT3G48430	REF6	0,99	7,56E-05
AT3G22780	TSO1	0,97	1,02E-03

AT2G36270	ABI5	0,89	3,55E-02
AT1G72830	HAP2C	0,83	3,30E-02
AT4G35900	FD	0,82	2,93E-02
AT4G19990	FRS1	0,80	2,39E-02
AT3G26744	SCRM	0,80	3,58E-03
AT3G48440		0,78	4,53E-02
AT2G19810	AT2G19810	0,77	1,16E-02
AT5G60450	ARF4	0,75	2,25E-02
AT5G56270	WRKY2	0,67	2,54E-02
AT1G68920	BHLH49	0,65	3,79E-02
AT3G04670	WRKY39	0,65	2,61E-02

Differential gene expression analysis of *slim1* mutants under 2D conditions revealed that many transcription factors were significantly upregulated.

The genes belonging to the NAC family, including ANAC085 (Log2 FC: 4.89), ANAC044 (Log2 FC: 4.72), ANAC103 (Log2 FC: 3.30), and ANAC13 (Log2 FC: 1.00), were significantly upregulated under this condition. Additionally, the WRKY family genes, such as WRKY14 (Log2 FC: 1.44), WRKY60 (Log2 FC: 1.35), WRKY2 (Log2 FC: 0.67), and WRKY39 (Log2 FC: 0.65), also exhibited increased expression under this condition.

The BHLH23 (Log2 FC: 2.42) and BHLH49 (Log2 FC: 0.65) genes, which are part of the bHLH family, were upregulated. Additionally, important transcription factors such as STOP2 (Log2 FC: 1.69), GRF8 (Log2 FC: 1.69), and HDT1 (Log2 FC: 1.38) were also upregulated. Furthermore, the REV (Log2 FC: 1.30), ERF055 (Log2 FC: 1.25), ABI5 (Log2 FC: 0.89), and SCRM (Log2 FC: 0.80) genes were similarly upregulated.

These results indicate that *slim1* mutants under 2B conditions exhibit changes in the transcriptional regulatory processes related to energy metabolism, stress responses, and gene expression regulation.

Table 4. 13. Down-regulated transcription factors in *slim1* mutants under 2B condition

AGI ID	Gene Name	log2 FC	P-value
AT2G26150	HSFA2	-5,76	4,55E-07
AT5G07700	MYB76	-4,57	1,12E-03
AT5G07690	MYB29	-3,42	7,36E-11
AT3G22100	BHLH117	-2,96	1,90E-02
AT2G40750	WRKY54	-2,34	2,43E-06
AT1G73870	COL7	-1,85	9,07E-06
AT4G06746	RAP2-9	-1,7	1,49E-04
AT5G17490	RGL3	-1,61	4,03E-04
AT5G01380	GT-3A	-1,6	1,63E-02
AT2G40340	DREB2C	-1,52	3,33E-05
AT2G42380	BZIP34	-1,51	1,93E-06
AT5G62430	CDF1	-1,47	4,12E-02
AT1G12610	DREB1F	-1,43	7,79E-04
AT1G63030	DREB1E	-1,43	7,13E-03
AT3G21330	BHLH87	-1,42	7,85E-03
AT3G11020	DREB2B	-1,39	6,69E-03
AT3G06490	MYB108	-1,38	3,48E-02
AT3G50260	ERF011	-1,34	3,87E-05
AT4G16780	HAT4	-1,3	1,17E-04
AT3G58120	BZIP61	-1,29	4,82E-06
AT4G32800	ERF043	-1,29	2,66E-03
AT4G17810		-1,27	3,57E-04
AT5G08130	BIM1	-1,24	1,68E-04
AT1G33760	ERF022	-1,21	3,77E-03
AT4G17230	SCL13	-1,19	6,05E-04
AT5G52020	ERF025	-1,19	1,97E-02
AT4G25480	DREB1A	-1,19	3,07E-02
AT1G18330	RVE7 AT1G18330	-1,18	4,69E-04
AT3G25710	BHLH32	-1,18	3,03E-03
AT2G44840	ERF13	-1,16	2,62E-03
AT4G31800	WRKY18	-1,15	2,68E-03
AT2G43010	PIF4	-1,13	2,44E-05
AT1G06040	BBX24	-1,12	1,17E-05
AT1G77640	ERF013	-1,12	1,50E-02
AT2G39250	SNZ	-1,11	3,41E-02
AT5G57390	AIL5	-1,1	3,15E-02
AT5G05410	DREB2A	-1,08	1,91E-04
AT1G13260	RAV1	-1,06	1,87E-05

AT5G57660	COL5	-1,05	7,85E-04
AT3G47620	TCP14	-1,02	1,42E-04
AT5G60890	MYB34	-1,01	3,51E-02
AT2G18300		-0,99	1,39E-03
AT2G02070	IDD5	-0,94	2,82E-04
AT5G44260	TZF5	-0,94	7,97E-03
AT4G17490	ERF6	-0,92	5,81E-04
AT4G17460	HAT1	-0,92	3,35E-03
AT1G21910	ERF012	-0,9	2,12E-02
AT4G39780	ERF060	-0,87	1,35E-02
AT2G46830	CCA1	-0,86	1,32E-02
AT1G33240	AT-GTL1	-0,85	7,37E-03
AT3G46640	PCL1	-0,85	1,37E-02
AT1G27730	ZAT10	-0,82	2,31E-03
AT5G08330	TCP21	-0,82	2,96E-02
AT3G06590	BHLH148	-0,81	1,76E-02
AT1G14580		-0,81	1,99E-02
AT3G15510	NAC056	-0,79	3,38E-02
AT5G60850	DOF5.4	-0,76	1,60E-02
AT5G47370	HAT2	-0,75	3,11E-03
AT3G55980	SZF1	-0,75	5,49E-03
AT2G38470	WRKY33	-0,75	3,50E-02
AT5G61590	ERF107	-0,75	3,68E-02
AT5G48250	COL10	-0,72	2,61E-02
AT1G50640	ERF3	-0,71	3,53E-02
AT1G80840	WRKY40	-0,71	4,68E-02
AT1G78600	LZF1	-0,7	3,33E-02
AT2G22430	ATHB-6	-0,67	1,22E-02
AT5G04340	ZAT6	-0,62	3,51E-02
AT1G61730		-0,62	4,38E-02
AT5G13180	NAC083	-0,61	4,79E-02

Differential gene expression analysis of *slim1* mutants under 2B condition revealed that many transcription factors were significantly downregulated.

NAC083 (Log2 FC: -0.61) and NAC056 (Log2 FC: -0.79) genes from the NAC family were suppressed under this condition. MYB76 (Log2 FC: -4.57), MYB29 (Log2 FC: -3.42), MYB108 (Log2 FC: -1.38) and MYB34 (Log2 FC: -1.01) genes from the MYB family were also significantly downregulated.

Among these repressed transcription factors are WRKY54 (Log2 FC: -2.34), WRKY18 (Log2 FC: -1.15), WRKY33 (Log2 FC: -0.75) and WRKY40 (Log2 FC: -0.71) genes from the WRKY family. Genes such as DREB2C (Log2 FC: -1.52), DREB1F (Log2 FC: -1.43), DREB1E (Log2 FC: -1.43) and DREB2B (Log2 FC: -1.39) from the DREB family were also downregulated. Transcription factors belonging to the ERF family such as ERF011 (Log2 FC: -1.34), ERF043 (Log2 FC: -1.29), ERF6 (Log2 FC: -0.92) and ERF3 (Log2 FC: -0.71) are also among those suppressed under this condition.

In addition, BHLH117 (Log2 FC: -2.96), BHLH87 (Log2 FC: -1.42), BHLH32 (Log2 FC: -1.18) and BHLH148 (Log2 FC: -0.81) genes belonging to the bHLH family were also observed to be significantly downregulated. These results suggest that *slim1* mutants show significant changes in the regulation of transcription factors under the 2B condition.

4.7 Heat Map of the 50 Most Variable Genes Of *slim1* Mutants and Control Groups Exposed to B Toxicity

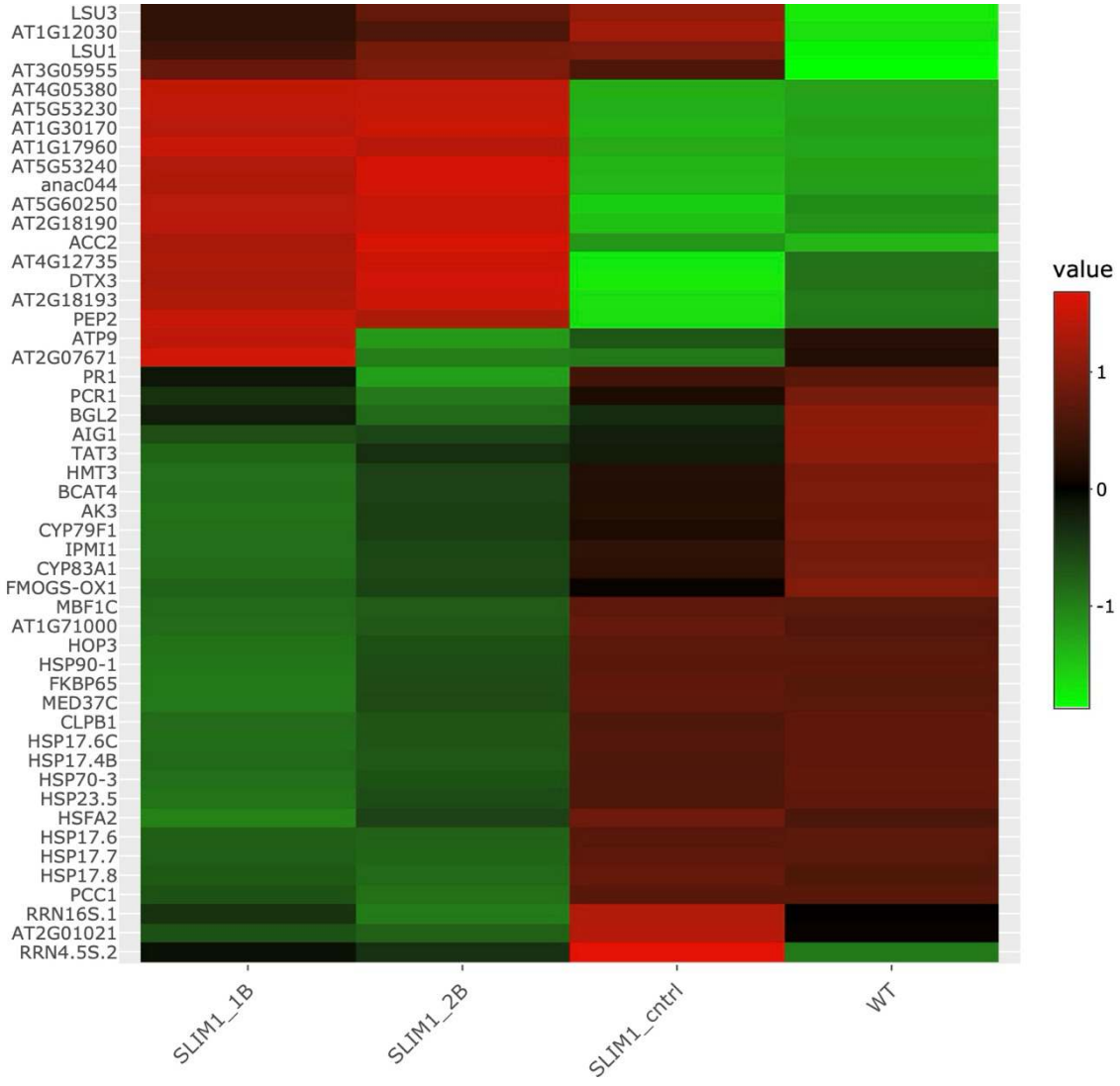

Figure 4. 9. Heat map showing gene expression profiles of *slim1* mutants and WT under different conditions

Heatmap analysis results show the 50 most variable genes in gene expression profiles between 1B and 2B conditions, control condition and WT group of *slim1* mutant. According to color coding, red colors indicate up-regulation and green colors indicate down-regulation.

It is observed that genes belonging to HSP family (e.g. HSP17.6C, HSP23.5, HSFA2) are excessively suppressed in *slim1* mutants exposed to B toxicity. It is noteworthy that these genes have higher expression levels in WT and *slim1* control conditions, but their expressions are significantly reduced in *slim1* mutants in 1B and especially 2B conditions.

In addition, it was observed that transcription factors such as anac044 were up-regulated in 1B and 2B conditions. In addition, some genes showing high expression in WT control and *slim1* control conditions are suppressed in *slim1* mutants exposed to B toxicity. For example, PR1, BGL2, and HSFA2 genes were overrepressed in 1B and 2B conditions.

These results suggest that *slim1* mutants develop condition-specific responses in gene expression regulation against B toxicity, and that suppression of the HSP gene family in particular may have a critical effect on adaptation processes to B stress.

4.8 Expression Profile of GSH-Related Genes of *slim1* Mutants under B Toxicity

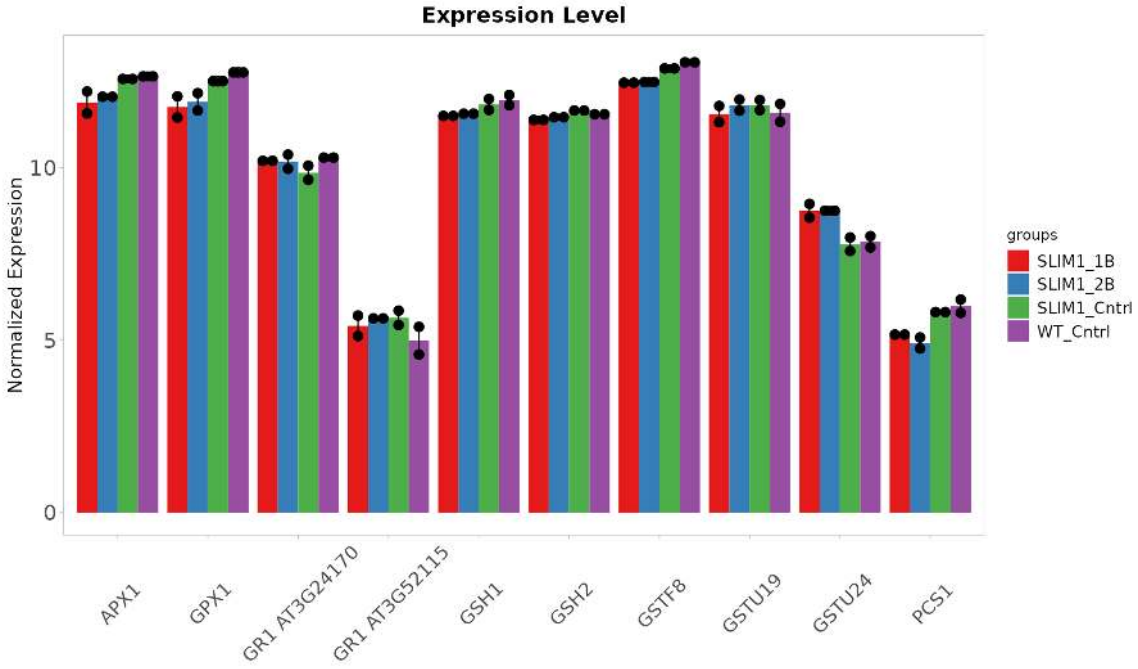

Figure 4. 10. Expression profile of GSH-related genes of *slim1* mutants under B toxicity

In the expression profile graph of genes that are effective in GSH processes, significant changes were observed in some genes compared to the control groups (*slim1* control and WT control). *APX1* and *GPX1* genes were found to have decreased expression levels in *slim1* mutants in 1B and 2B conditions compared to control groups. There was an increase in *GR1* genes, especially in the AT3G52115 gene, in mutants compared to wild type control. On the other hand, more stable expression levels were observed for AT3G24170.

Although slight decreases were observed in the *GSH1* and *GSH2* genes, they generally remained stable. While there was a decrease in the expression levels of *GSTF8* and *GSTU19* genes compared to the control groups, an increase was observed in *GSTU19* in the

2B condition compared to the wild type. An increase was detected in *GSTU24* gene in 1B and 2B conditions. The expression levels of the *PCS1* gene were reduced compared to the WT control group.

4.9 Expression Profile of Anthocyanin-Related Genes of *slim1* Mutants under B Toxicity

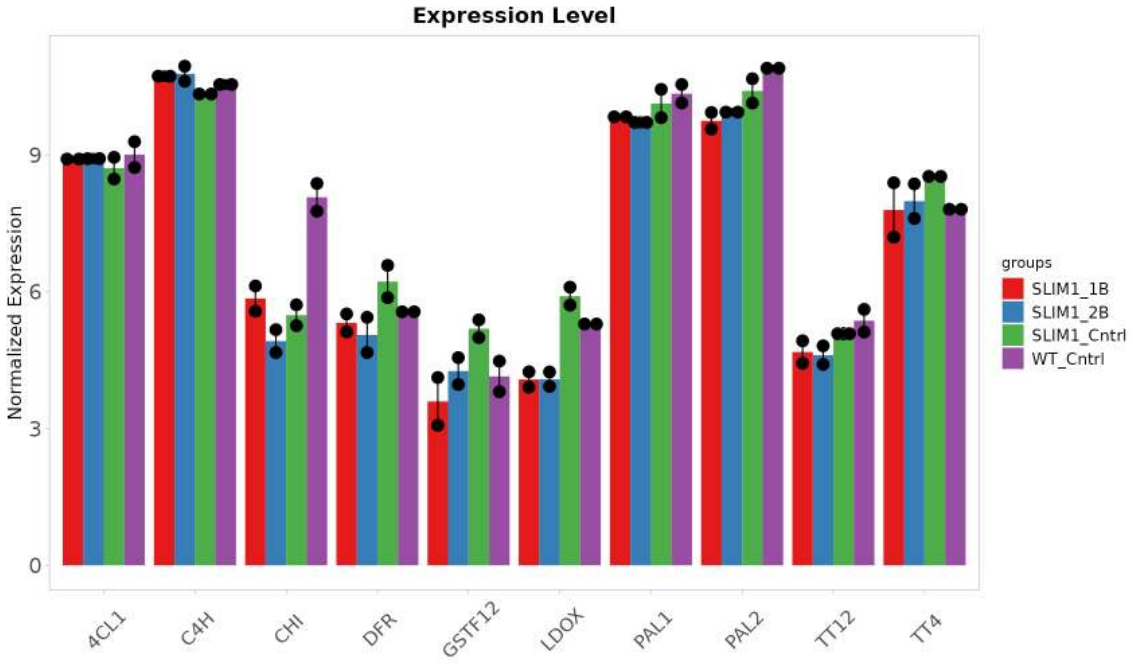

Figure 4. 11. Expression profile of anthocyanin-related genes of *slim1* mutants under B toxicity

In the expression profiles of genes involved in anthocyanin biosynthesis pathways, the expression levels of *4CL1* and *C4H* genes showed a significant increase in *slim1* mutants compared to both *slim1* control group and WT control group in 1B and 2B conditions. Since these genes play an active role in the early steps of anthocyanin biosynthesis, this increase under toxicity conditions can be considered an adaptive response. A significant decrease was observed in *CHI* gene in 1B and 2B conditions compared to slim1 control and WT control group. A significant decrease was observed in *DFR* gene especially in 2B conditions compared to all other groups. A significant decrease was seen in the *GSTF12* gene in 1B and 2B conditions compared to the WT control group and the *slim1* control. *LDOX(ANS)* gene showed a significant decrease in *slim1* mutants exposed to B toxicity. The *PAL1*, and *PAL2* genes showed a generally stable expression profile. However, it was observed that the *PAL1*

gene was at similar levels to the WT control group and was less affected by the toxicity conditions. *TT12* and *TT4(CHS)* genes showed slight decreases in 1B and 2B conditions compared to the WT control group and the *slim1* control group.

4.10 Expression Profile of B and Anthocyanin Transport-Related Genes of *slim1* Mutants under B Toxicity

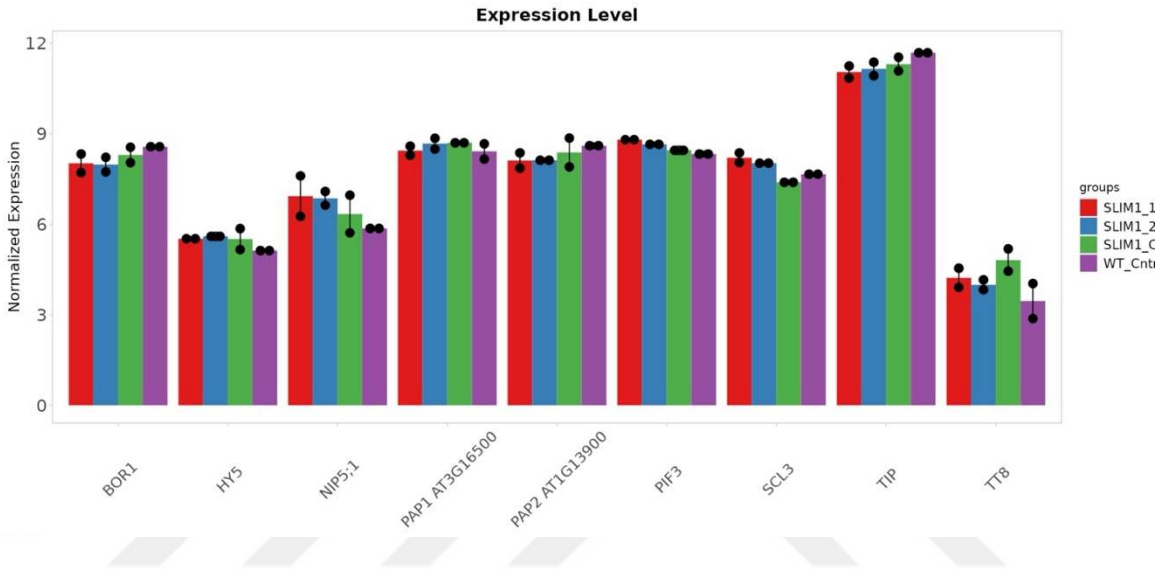

Figure 4. 12. Expression profile of anthocyanin transport-related genes of *slim1* mutants under B toxicity

Looking at the expression profile of genes related to anthocyanin transport and processes, there was a slight decrease in the *BOR1* gene, especially in mutants, but no significant change was observed. In the *HY5* gene, a slight increase was observed in under 1B and 2B conditions compared to the *slim1* control and WT control. An increase was observed in the *NIP5;1* gene in *slim1* mutants compared to the WT control group, especially in the 1B and 2B conditions. Expression levels of *PAP1* (AT3G16500) and *PAP2* (AT1G13900) genes showed a stable course compared to control groups. A significant increase in the *PIF3* gene was observed in 1B and 2B conditions compared to the control groups. Similarly, an increase was observed in the *SCL3* gene in under 1B and 2B conditions compared to the WT and *slim1* mutant control groups. A significant decrease was observed in the *TIP* gene compared to the control groups, especially in the 1B and 2B conditions. A significant decrease in the *TT8* gene was detected in all conditions compared to the *slim1* mutant control. However, an increase was observed when compared with WT.

4.11 Expression Profile of Glucosinolate (GSL) Biosynthesis-Related Genes of *slim1* Mutants under B Toxicity

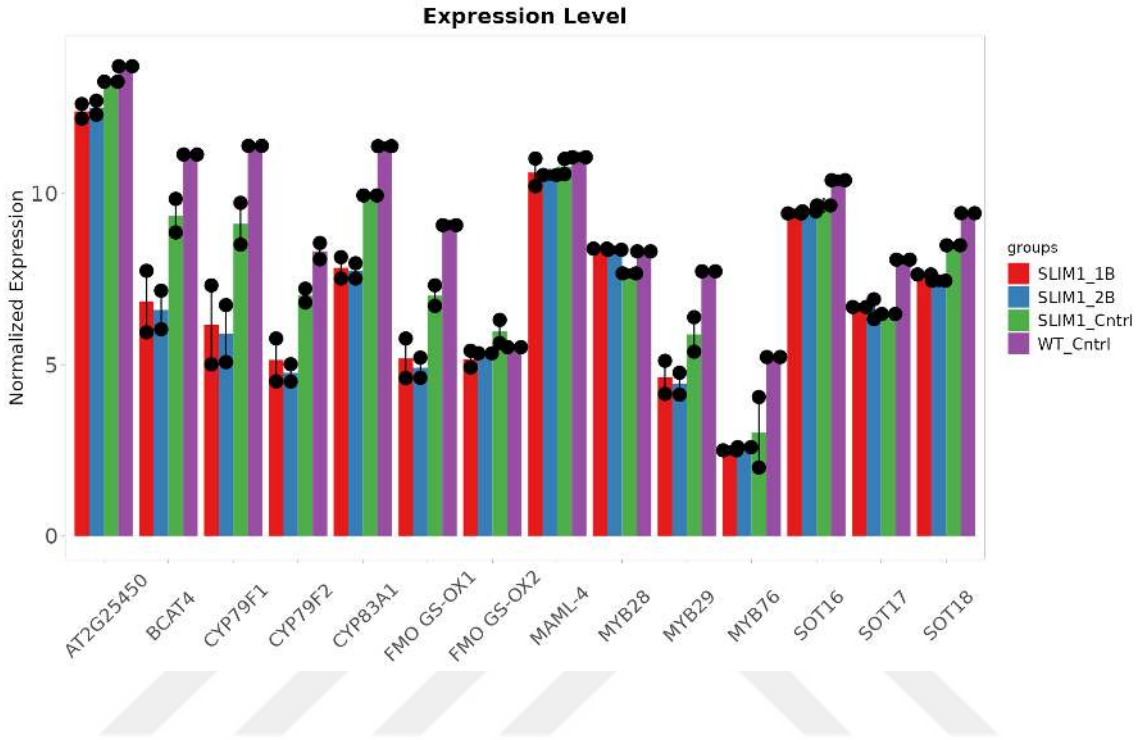

Figure 4. 13. Expression profile of glucosinolate (GSL) biosynthesis-related genes of *slim1* mutants under B toxicity

In the GSL biosynthesis gene expression analysis performed under 1B and 2B conditions in *slim1* mutants, significant changes were observed in some genes compared to the control groups (*slim1* control and WT control). Expression levels in *MAML-4, MYB28*, and *FMO GS-OX2* genes remained stable compared to the control groups. A significant decrease in expression levels was observed in *BCAT4, CYP79F1, CYP79F2, CYP83A1*, and *MYB29* genes compared to the WT control group, especially in the slim1 mutant under 2B condition. Similarly, a decrease was observed in *SOT16, SOT17*, and *SOT18* genes compared to the WT control group and the slim1 control group. A slight increase was detected in AT2G25450 and *FMO GS-OX1* genes compared to the WT control group. These findings demonstrate changes in gene expression levels.

4.12 Expression Profile of Glucosinolate (GSL) Transport and Modification-Related Genes of *slim1* Mutants under B Toxicity

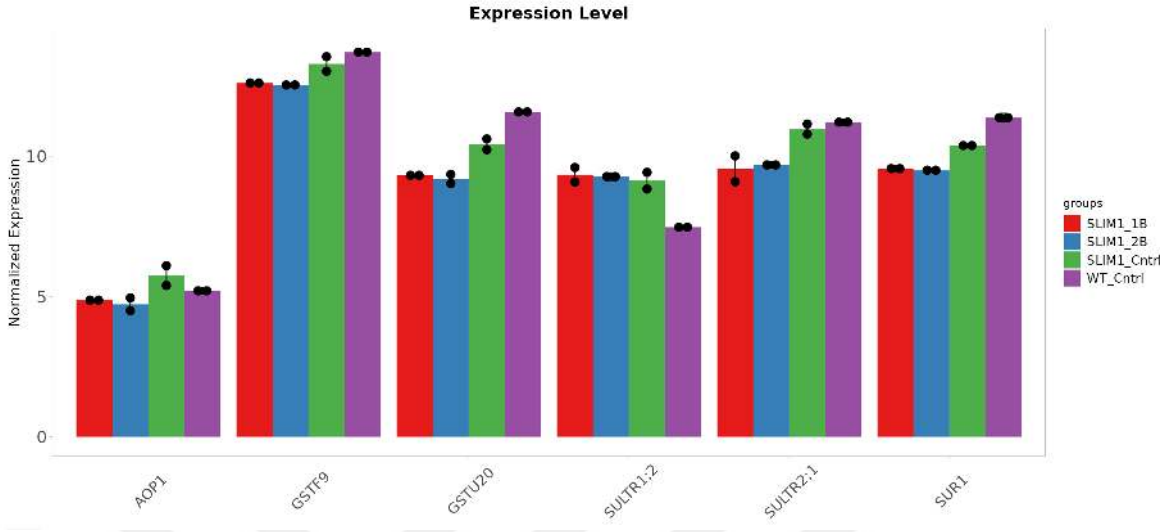

Figure 4. 14. Expression profile of glucosinolate (GSL) transport and modification- related genes of *slim1* mutants under B toxicity

In *slim1* mutants, significant changes were observed in the expression levels of genes responsible for GSL transport and modification under 1B and 2B conditions compared to the test groups (*slim1* test and WT test).

A significant decrease was observed in the *AOP1* gene in *slim1* mutants under 1B and 2B conditions compared to the WT test group. A significant decrease was detected in the *GSTF9* and *GSTU20* genes under both mutant conditions compared to the WT control. A significant increase was observed in the *SULTR1;2* gene in *slim1* mutants (especially in the 2B condition) compared to the WT control, while a significant decrease was observed in the *SULTR2;1* and *SUR1* genes under 1B and 2B conditions compared to the WT test group. These decreases were at similar levels in the 1B and 2B conditions.

These findings show that GSL transport and modification processes are rearranged under B toxicity and these processes are genetically adapted with different responses. In particular, the increase in the *SULTR1;2* gene can be considered as an adaptive response to sulfur transport, while the suppression in the *SULTR2;1* and *SUR1* genes indicates that transport mechanisms are limited under toxicity conditions.

4.13 Expression Profile of Glucosinolate (GSL) Modification and Catabolism-Related Genes of *slim1* Mutants under B Toxicity

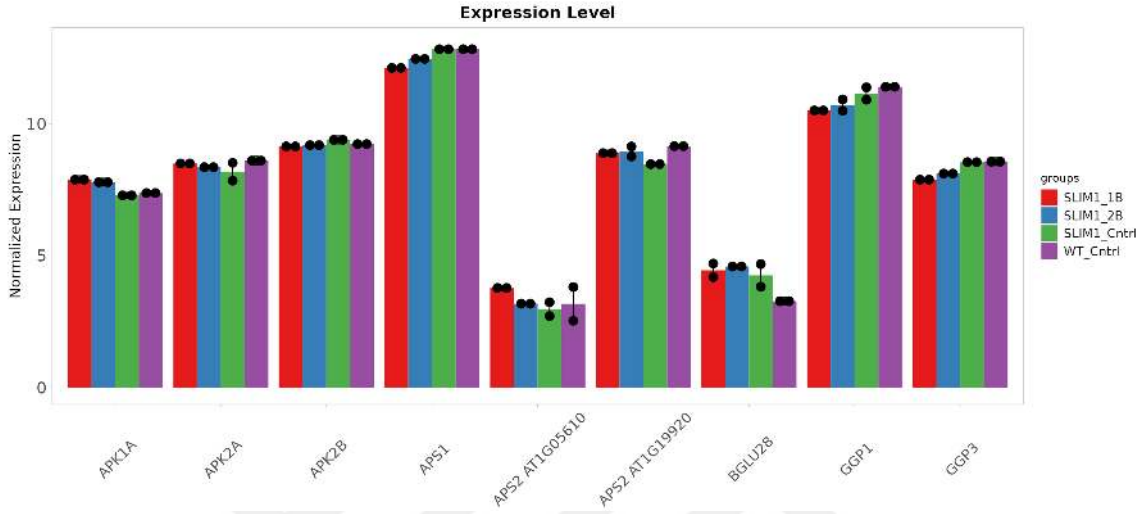

Figure 4. 15. Expression profile of glucosinolate (GSL) modification and catabolism-related genes of *slim1* mutants under B toxicity

In *slim1* mutants, the following changes were observed in the expression levels of genes related to GSL metabolism and catabolism under 1B and 2B conditions compared to the control groups (*slim1* control and WT control): A significant increase was observed in *APK1A, APK2A*, and *APK2B* genes in *slim1* mutants under 1B and 2B conditions compared to WT control.

Expression levels close to the WT control were detected in the *APS1* and *APS2* (AT1G06510 and AT1G09210) genes of *slim1* mutants in 1B and 2B conditions. A clear increase in the BGLU28 gene was observed in 1B and 2B conditions of *slim1* mutants compared to the WT control. A slight decrease was detected in GGP1 and GGP3 genes in slim1 mutants under 1B and 2B conditions compared to WT control.

4.14 Regulation Of Flavonoid Biosynthesis In Slim1 Mutants Under B Toxicity

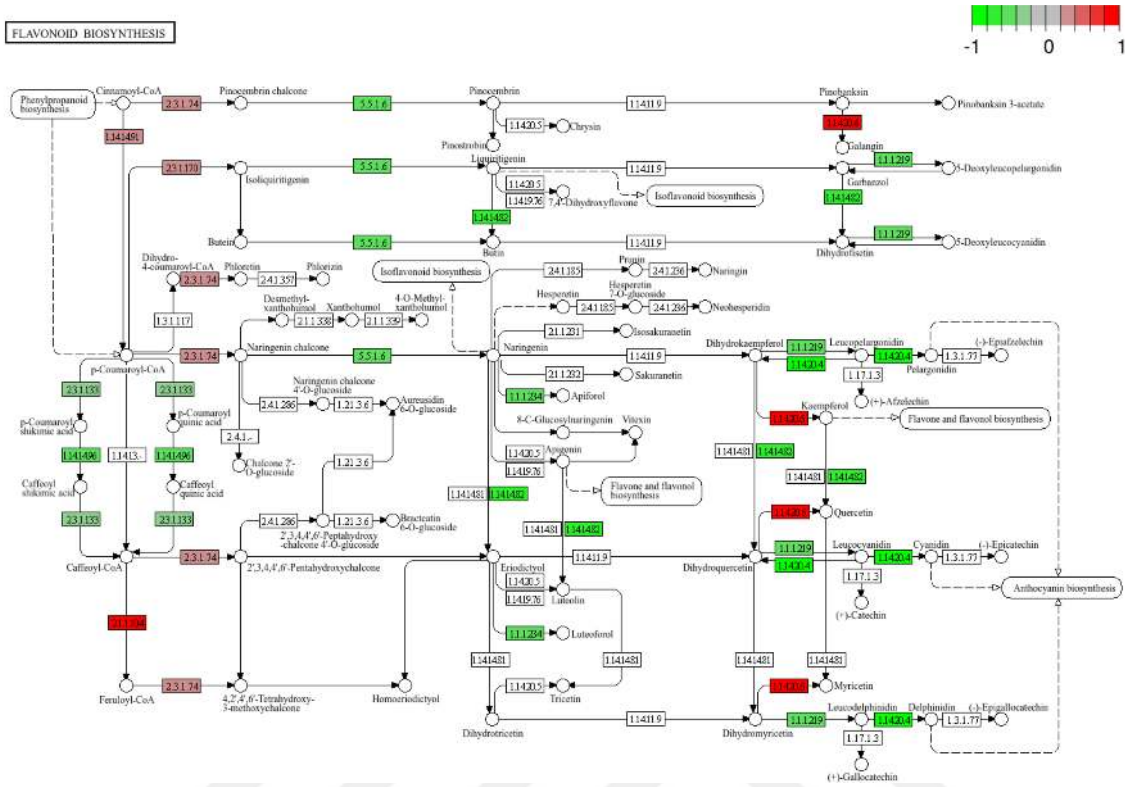

Figure 4. 16. Map showing gene expression changes in the flavonoid biosynthesis pathway of the *slim1* mutant under 2B condition

This KEGG pathway map illustrates how flavonoid biosynthesis pathways are modified under B toxicity in *slim1* mutants. The analysis revealed that genes highlighted in green were suppressed and processes involved in producing intermediates like naringenin, luteolin, and apigenin were diminished. This indicates that certain flavonoid biosynthetic pathways were suppressed for the sake of energy conservation. In contrast, genes highlighted in red were upregulated, leading to an increase in the synthesis of compounds such as kaempferol and quercetin. This rise suggests that the antioxidant defense and stress tolerance mechanisms of flavonoids are prioritized in response to B toxicity. These findings indicate that flavonoid biosynthesis pathways undergo adaptive regulation in *slim1* mutants.

4.15 Top Five Up-Regulated Genes Identified in the *slim1* Control Group

Table 4. 14. Top five up-regulated genes identified in the *slim1* control group

AGI ID	Gene Name	Description	Log2FC	P-value
AT3G05955	AT3G05955	None	-7,1	1,32E-04
AT3G49580	LSU1	Response To Low Sulfur Gene Family Member; Expressed During Sulfur Deficiency	-6,84	2,03E-18
AT1G12030	AT1G12030	DUF506 gene family member	-5,04	2,91E-28
AT3G49570	LSU3	Response To Low Sulfur Gene Family Member; Expressed During Sulfur Deficiency	-4,95	4,65E-28
AT5G26220	GGCT2;1	ChaC-like family protein	-4,13	5,23E-30

The five most expressed genes in the *slim1 c*ontrol group were identified.Among these genes, AT3G05955 (Log2 FC: -7.1; P-Value: 1.32E-04) ranks first and has no defined function yet. LSU1 (AT3G49580, Log2 FC: -6.84; P-Value: 2.03E-18) and LSU3 (AT3G49570, Log2 FC: -4.95; P-Value: 4.65E-28) are members of the gene family that responds to low sulfur conditions and are known to be expressed during sulfur deficiency. AT1G12030 (Log2 FC: -5.04; P-Value: 2.91E-28) is a member of the DUF506 gene family. GGCT2;1 (AT5G26220, Log2 FC: -4.13; P-Value: 5.23E-30) was identified as a gene belonging to the ChaC-like protein family.

4.16 Top Five Down-Regulated Genes Identified in the *slim1* Control Group

Table 4. 15. Top five down-regulated genes identified in the *slim1* control group

AGI ID	Gene Name	Gene Name	Log2FC	P-value
AT1G33960	AIG1	Identified as a gene that is induced by avirulence gene avrRpt2 and RPS2 after infection with Pseudomonas syringae pv maculicola strain ES4326 carrying avrRpt2	2,96	1,55E-05
AT2G47550	PME20	Pectin methylesterase inhibitor; INVI-PMEI protein isoform which clusters with a PME.	2,85	1,49E-02
AT5G13320	PBS3	An important enzyme in defense signaling that facilitates the conjugation of 4-HBA and pAPA with amino acids. It is necessary for the accumulation of salicylic acid and the activation of defense responses.	2,84	2,27E-03
AT4G37990	CAD8	Encodes NADP+ oxidoreductase, an aromatic alcohol whose mRNA levels increase in response to treatment with various phytopathogenic bacteria.	2,8	4,65E-02
AT2G43570	CHI	Putative basic chitinase.	2,8	4,28E-07

The five most suppressed genes in the *slim1* control group were identified. Among these, AIG1 (AT1G33960, Log2 FC: 2.96; P-Value: 1.55E-05) stands out as a gene induced after infection with *Pseudomonas syringae* and plays a significant role in plant defense mechanisms. PME20 (AT2G47550, Log2 FC: 2.85; P-Value: 1.49E-02), a pectin methylesterase inhibitor, has been linked to cell wall dynamics. PBS3 (AT5G13320, Log2 FC: 2.84; P-Value: 2.27E-03) is a crucial enzyme that plays a vital role in activating defense responses and accumulating salicylic acid.

Other suppressed genes include CAD8 (AT4G37990, Log2 FC: 2.8; P-Value: 4.65E-02), which has NADP+ oxidoreductase activity and is involved in responses against phytopathogenic bacteria. CHI (AT2G43570, Log2 FC: 2.8; P-Value: 4.28E-07) is categorized as a potential basic chitinase associated with pathogen responses.

4.17 Top Five Up-Regulated Genes Identified in *slım1* 1B Mutants Under B Toxicity

Table 4. 16. Top five up-regulated genes identified in *slim1* 1b mutants under B toxicity

AGI ID	Gene Name	Gene Name	Log2FC	P-value
AT3G05955	AT1G08117	None	-6,84	2,67E-05
AT2G21450	CHR34	Chromatin remodeling	-5,17	3,12E-02
AT5G14490	ANAC085	NAC domain containing protein	-5,11	3,39E-03
AT1G71280	RH55	DEA(D/H)-box RNA helicase family protein	-5,07	3,29E-02
AT3G49580	LSU1	Response To Low Sulfur Gene Family Member; Expressed During Sulfur Deficiency	-4,9	1,47E-09

The five most expressed genes in *slim1* 1B mutants exposed to B toxicity have been identified. Among these genes, AT1G08117 (Log2 FC: -6.84; P-Value: 2.67E-05) ranks first but has yet to be defined functionally. CHR34 (AT2G21450, Log2 FC: -5.17; P-Value: 3.12E-02) stands out as a gene associated with chromatin remodeling processes. ANAC085 (AT5G14490, Log2 FC: -5.11; P-Value: 3.39E-03) encodes a protein containing an NAC domain and is linked to gene regulation and stress responses.

Additionally, RH55 (AT1G71280, Log2 FC: -5.07; P-Value: 3.29E-02), a protein belonging to the RNA helicase family, plays a significant role in RNA editing and metabolism processes. LSU1 (AT3G49580, Log2 FC: -4.9; P-Value: 1.47E-09), a member of the gene family that responds to low sulfur conditions, is expressed during sulfur deficiency.

4.18 Top Five Down-Regulated Genes Identified in *slım1* 1B Mutants Under B Toxicity

Table 4. 17. Top five down-regulated genes identified in *slim1* 1b mutants under B toxicity

AGI ID	Gene Name	Gene Name	Log2FC	P-value
AT1G71000	AT1G71000	Chaperone DnaJ-domain superfamily protein	8,01	2,09E-26
AT5G12030	HSP17.6	Encodes a cytosolic small heat shock protein with chaperone activity that is induced by heat and osmotic stress and is also expressed late in seed development.	7,22	2,84E-16
AT5G12020	HSP17.6II	17.6 kDa class II heat shock protein	6,44	2,65E-27
AT3G12580	HSP70	Cytoplasmically localized member of the heat shock protein 70 family. Forms complex with J3. Mutants are heat sensitive and show increased accummulation of insoluble proteins suggesting a role in thermotolerance.	6,01	2,74E-10
AT1G53540	HSP17.6C	Member of the class I small heat-shock protein (sHSP) family, which accounts for the majority of sHSPs in maturing seeds	5,79	4,92E-31

The five most repressed genes were identified in *slim1* 1B mutants exposed to B toxicity. Among these genes, AT1G71000 (Log2 FC: 8.01; P-Value: 2.09E-26) stands out as a DnaJ-domain superfamily protein with chaperone activity. HSP17.6 (AT5G12030, Log2 FC: 7.22; P-Value: 2.84E-16) and HSP17.6II (AT5G12020, Log2 FC: 6.44; P-Value: 2.65E-27) are small heat shock proteins (sHSPs) that are induced under heat and osmotic stress and expressed during seed development.

HSP70 (AT3G12580, Log2 FC: 6.01; P-Value: 2.74E-10) is a cytoplasmic heat shock protein that has an important role in thermotolerance mechanisms. HSP17.6C (AT1G53540, Log2 FC: 5.79; P-Value: 4.92E-31) belongs to the family of small heat shock proteins and constitutes the majority of these proteins in maturing seeds.

4.19 Top Five Up-Regulated Genes Identified in *slim1* 2B Mutants Under B Toxicity

Table 4. 18. Top five up-regulated genes identified in *slim1* 2b mutants under B toxicity

AGI ID	Gene Name	Description	Log2FC	P-value
AT3G05955	AT3G05955	None	-5,17	6,72E-05
AT3G49580	LSU1	Response To Low Sulfur Gene Family Member; Expressed During Sulfur Deficiency	4,98	5,97E-11
AT2G21450	CHR34	Chromatin remodeling	-4,89	4,00E-02
AT5G14490	ANAC085	NAC domain containing protein	-4,82	5,54E-03
AT1G71280	RH55	DEA(D/H)-box RNA helicase family protein	4,8	4.58e-02

The five most highly expressed genes in *slim1* 2B mutants exposed to B toxicity were identified. AT3G05955 (Log2 FC: -5.17; P-Value: 6.72E-05), whose function remains undefined, stands out as one of the most expressed genes in this group. LSU1 (AT3G49580, Log2 FC: 4.98; P-Value: 5.97E-11) is a member of the gene family that responds to low sulfur conditions and is expressed during sulfur deficiency. CHR34 (AT2G21450, Log2 FC: -4.89; P-Value: 4.00E-02) was identified as a gene associated with chromatin remodeling processes. ANAC085 (AT5G14490, Log2 FC: -4.82; P-Value: 5.54E-03) is a transcription factor containing an NAC domain and plays an important role in stress responses. RH55 (AT1G71280, Log2 FC: 4.8; P-Value: 4.58E-02), a protein belonging to the RNA helicase family, is involved in RNA regulation and metabolic processes.

4.20 Top Five Down-Regulated Genes Identified in *slim1* 2B Mutants Under B Toxicity

Table 4. 19. Top five down-regulated genes identified in *slim1* 2b mutants under B toxicity

AGI ID	Gene Name	Description	Log2FC	P-value
AT1G71000	AT1G71000	Chaperone DnaJ-domain superfamily protein	9,15	1,42E-28
AT5G12030	HSP17.6	Encodes a cytosolic small heat shock protein with chaperone activity that is induced by heat and osmotic stress and is also expressed late in seed development.	8,2	2,30E-22
AT5G12020	HSP17.6II	17.6 kDa class II heat shock protein	7,73	4,56E-33
AT2G14610	PR1	PR1 gene expression is induced in response to a variety of pathogens. It is a useful molecular marker for the SAR response.	6,95	8,23E-07
AT1G14880	PCR1	Plant Cadmium Resistance	6,8	5,37E-32

The five most repressed genes were identified in *slim1* 2B mutants exposed to B toxicity. AT1G71000 (Log2 FC: 9.15; P-Value: 1.42E-28), a DnaJ-domain superfamily protein with chaperone activity, ranks among the genes with the highest levels of repression. HSP17.6 (AT5G12030, Log2 FC: 8.2; P-Value: 2.30E-22) and HSP17.6II (AT5G12020, Log2 FC: 7.73; P-Value: 4.56E-33) are small heat shock proteins (sHSPs) involved in stress responses and seed development.

PR1 (AT2G14610, Log2 FC: 6.95; P-Value: 8.23E-07) functions as an important molecular marker for the systemic acquired resistance (SAR) response and is induced by various pathogens. PCR1 (AT1G14880, Log2 FC: 6.8; P-Value: 5.37E-32) has been identified as a gene regulating cadmium resistance in plants.

5. DISCUSSION

The study of *slim1* mutants and their behavior under B toxicity is crucial for understanding how plants adapt to environmental stresses. *Slim1* serves as a key transcription factor in regulating sulfur metabolism, and the response of mutants to stressful environmental conditions may influence genetic regulation processes. *Slim1* mutants, particularly those affected by B toxicity, display distinct genetic profiles, highlighting adaptation mechanisms to this stressor.

The PCA (Principal Component Analysis) analysis clearly shows the genetic responses of the *slim1* 1B, *slim1* 2B, control, and WT groups to B toxicity and the distinctions between them. The analysis reveals that each condition shows a unique genetic variation, and the groups exposed to B toxicity do not show significant differences.

The *slim1* control group, although not exposed to B toxicity, is clearly separated from the WT group. This separation indicates that the slim1 control group, being a mutant, has developed different genetic regulations in sulfur metabolism and other regulatory processes compared to WT. However, it also separates from the *slim1* 1B and *slim1* 2B groups since it was not exposed to B toxicity. On the other hand, the distant clustering of the control group reflects the intrinsic genetic diversity of the group and variations independent of other environmental effects. The WT group was located relatively close to the control group in the PCA plot, indicating that WT exhibits similar stability to the control group in terms of genetic regulations. However, the narrower range of variation in WT suggests that this group has a more consistent structure in genetic regulation, and its natural variations, apart from environmental stress responses, are limited.

The *slim1* 1B group is one that is exposed to B toxicity and is distinctly separated from both the control and WT groups in the PCA plot. The broader variation observed in *slim1* 1B suggests that this group has developed versatile adaptation mechanisms to manage B toxicity. In GO analyses, various cellular adaptation processes, including ribosome biogenesis, RNA processing, and protein synthesis, were found to be more active in Slim1 1B. This genetic variation indicates that Slim1 1B possesses a more flexible response to toxicity.

The *slim1* 2B group is another group exposed to B toxicity, and although it is situated close to *slim1* 1B in the PCA graph, it demonstrates a narrower genetic variation. This indicates that *slim1* 2B has developed a more specific regulatory mechanism against B toxicity. In GO analyses, it was found that sulfur metabolism, chromatin rearrangement, and RNA processing were particularly prominent in slim1 2B, while processes such as photosynthesis and energy metabolism were suppressed. This focused genetic response of *slim1* 2B illustrates that resources are directed more strategically against toxicity.

Another notable aspect of the PCA analysis is the clustering of the slim1 1B and *slim1* 2B groups in close proximity to each other. This suggests that both groups have developed a response to B toxicity via shared biological processes. However, the broader variation within *slim1* 1B indicates that this group has established more versatile regulations against toxicity. In contrast, *slim1* 2B demonstrates a narrower and more targeted adaptation.

The analysis of Venn diagrams highlights the genetic adaptations developed by *slim1* mutants in response to B toxicity and the variations of these adaptations across different genotypes. The Venn diagram of up-regulated genes indicates that there are 241 common genes between the *slim1* 1B and *slim1* 2B mutants. These common genes reflect the fundamental adaptation mechanisms established by both mutants against B toxicity. The fact that 80 genes in the *slim1* 1B group are up-regulated solely in this mutant suggests that this group has developed a more comprehensive adaptation strategy. It is believed that these genes particularly contribute to processes such as ribosome biogenesis and protein production. Furthermore, the presence of 78 genes that are exclusively up-regulated in the *slim1* 2B group indicates that this mutant has developed an adaptation strategy focused on binding activities and energy conservation. These findings demonstrate that despite the common regulation of similar processes in both mutants against B toxicity, they exhibit genotype-specific adaptations differences.

The Venn diagram illustrating the up-regulated genes of wt and slim1 mutants reveals that the *slim1* 1B and slim1 2B mutants share 370 common genes and exhibit similar responses to B toxicity. However, only 16 genes were up-regulated across all conditions, indicating that general adaptation mechanisms against B toxicity occur within a limited genetic framework. In the *slim1* 1B group, 169 genes were up-regulated only in this mutant, suggesting that this group has undergone more extensive genetic regulation, while in the *slim1* 2B group, 122 genes were up-regulated, suggesting that this group has developed a more specific adaptation mechanism. The fact that only 2-4 genes were up-regulated in

common between the *slim1* control group and wt indicates that the mutant groups are largely distinct from wt and that slim1 plays a regulatory role against B toxicity.

The Venn diagram of down-regulated genes shows that there are 915 common genes between *slim1* 1B and *slim1* 2B mutants. These genes reveal the basic regulation mechanisms shared by the two mutants against B toxicity. However, 277 genes specific to slim1 1B and 235 genes specific to slim1 2B were down-regulated, indicating that both mutants have developed different regulation mechanisms against B toxicity. Only 20 genes were down-regulated in the slim1 control group, indicating that the genetic profile of this group not exposed to B toxicity is significantly different from the other mutants.

Another down-regulation analysis compared with the Slim1 control group showed that a total of 421 genes were down-regulated in the *slim1* 1B and *slim1* 2B groups. These common genes indicate that the two mutants regulate similar biological processes against B toxicity. However, 144 genes specific to *slim1* 1B and 124 genes specific to *slim1* 2B were down-regulated, clearly indicating that both mutants respond to B toxicity with different strategies.

To understand the critical role of *slim1* in adaptation to B toxicity, RNA-Seq analysis was performed from *Arabidopsis thaliana* leaves to identify differentially expressed genes (DEGs). These genes were ranked according to p-values using biological processes, molecular functions and KEGG pathways. The analyses revealed that *slim1* affected gene expression profiles and associated metabolic processes in groups exposed to different B concentrations.

As a result of GO analyses, it was observed that in the *slim1* control group, the response to cellular sulfur deficiency and sulfate transport processes were up-regulated. This supports the regulatory role of *slim1* in sulfur metabolism. Kayihan et al. [87] reported that sulfur transport genes were up-regulated at the transcriptional level and were effective in maintaining ion homeostasis against B toxicity. Similarly, in the study conducted by Piłsyk and Paszewski [88], the regulatory effect of S *slim1* on sulfate transporter activities was emphasized. An increase in the activation of sulfur metabolism was observed in *slim1* 1B and 2B groups. It was stated by Harun et al. [89] that *slim1* supports sulfur metabolism by activating sulfur transporter genes in response to B toxicity. In addition, metabolic pathways

such as glucosinolate biosynthesis were suppressed in all mutants. The suppressive effect of *slim1* mutants on glucosinolate biosynthesis is considered as one of the genetic strategies of plants to adapt to stress conditions. Literature shows that *slim1* acts as a central regulator of sulfur metabolism and allows the diversion of energy and resources to essential processes by suppressing glucosinolate biosynthesis under sulfur deficiency conditions. [90]

Notably, ribosome biogenesis and rRNA processing are upregulated in the *slim1* 1B mutant. These processes enhance protein production and help the cell adapt to toxicity. Increased protein production is crucial for regenerating damaged proteins and maintaining cellular functions under toxic conditions [91]. Similarly, ribosome biogenesis and RNA processing were upregulated in the *slim1* 2B condition.

The findings of the study by Tombuloğlu et al. [92] provides insights into the regulatory effects of B toxicity on RNA processes. The increase observed in RNA processes under B toxicity in *slim1* mutants suggests that these mechanisms play a central role in adapting to toxic conditions. The absence of regulation by *slim1* may have led to this more pronounced increase in RNA processes. In the *slim1* 2B mutant, RNA binding, ATP binding, and polysaccharide binding activities were found to be elevated. This situation is viewed as an adaptive strategy for gene regulation and enhancing the stability of existing RNA. Notably, the rise in RNA binding activities indicates that post-transcriptional regulatory mechanisms are being reinforced.

The suppression of photosynthesis-related genes under *slim1*1B condition indicates that plants focus their energy away from intensive processes and on basic metabolic needs. The suppression of photosynthesis processes allows the redirection of cellular resources, especially by saving energy. In the study of Maruyama-Nakashita et al. [65], it was reported that the suppression of photosynthesis in environmental stress conditions such as sulfur deficiency allows resources to be directed to basic processes and increases the survival chance of plants.

It was observed that photosynthesis processes were similarly suppressed in the *slim1* 2B condition. The suppression of photosynthesis processes under B toxicity is widely seen in the literature [41]. In addition, the suppression of heat shock proteins in the *slim1* mutant under the 2B condition is also noteworthy. Heat shock proteins are critical for ensuring the folding and stability of proteins under stress conditions. The suppression of these proteins indicates that environmental stresses can disrupt protein homeostasis. Yang and Wang [93] reported that high-temperature stress negatively affects photosynthesis and protein stability by suppressing heat shock proteins. This suggests that the protein balancing capacity of the *slim1* mutant under the 2B condition may be limited.

The increase in binding activities observed under *slim1* 2B conditions can be seen as a significant adaptive response. This increase in binding activities facilitates the optimization of cellular functions, including the regulation of RNA metabolism and energy transport. Sirko and Wawrzyńska Alan [94] demonstrated that the increase in binding activities plays a crucial role in regulating molecular interactions in response to environmental stresses. This increase may serve as an indicator of *slim1* mutant under 2B conditions ability to maintain and regulate cellular functions under stress conditions.

When these findings are evaluated together, it can be concluded that *slim1* mutants develop different adaptation strategies against environmental stresses. While *slim1* conserves its resources by reducing energy consumption in the 1B condition, *slim1* optimizes cellular regulation processes by increasing binding activities in the 2B condition. However, the suppression of heat shock proteins suggests that *slim1* in the 2B condition may limit stress tolerance mechanisms. These findings provide an important basis for a better understanding of the genetic and metabolic adaptations of *slim1* mutants.

Genetic responses of *slim1* mutants to B toxicity were detailed by analyzing up- and down-regulated transcription factors. These TFs stand out as genetic regulators that play critical roles in plant adaptation to stress conditions. When we look at the up-regulated transcription factors, NAC family TFs (e.g. ANAC085, ANAC044, ANAC103) were up-regulated in response to abiotic stresses in *slim1* 1B and *slim1* 2B mutants. ANAC085 in particular was up-regulated at the highest level in both mutants. NAC TFs support stress adaptations by playing a role in cell wall remodeling and gene regulation [95].

However, WRKY family TFs (e.g. WRKY71, WRKY60, WRKY14) were upregulated in both *slim1* mutant 1B and 2B conditions, indicating activation of defense responses against B toxicity [96]. The upregulation of ribosome biogenesis and protein production-related TFs (e.g. STOP2, GRF8, HDT1) in the *slim1* 1B mutant suggests that this mutant has developed a broad adaptation strategy. In contrast, TFs focused on binding activities and energy conservation (e.g. ERF055, AZF1) were prominent in the slim1 2B mutant, indicating that *slim1* 2B prefers more specific regulatory mechanisms.

When the down-regulated TFs were examined, HSFA2 emerged as the most highly repressed factor in both mutants. The repression of HSFA2 may suggest that protein stabilization against B toxicity was insufficient [97]. Furthermore, the repression of MYB family transcription factors (e.g., MYB76, MYB29) implies that secondary metabolic processes, such as glucosinolate biosynthesis, were relegated to the background [98].

In the *slim1* 2B mutant, the repression of DREB family transcription factors (e.g., DREB2C, DREB1F, DREB1E) indicates that this mutant restricts water transport and osmotic stress responses. Likewise, the suppression of RAP2-9 and ERF011 transcription factors involved in ethylene signaling pathways signifies that hormonal responses to B toxicity are limited. In *slim1* mutant 1B condition, the repressed transcription factors are associated with growth and development rather than energy-intensive biosynthetic processes (e.g., PRE1, HAT4).

The analysis of the most up- and down-regulated genes provides important clues to understanding the genetic regulations developed by *slim1* mutants in response to B toxicity. The LSU1 gene was identified as one of the up-regulated genes across all mutant groups and was found to play a crucial role in sulfur metabolism. In the *slim1* 1B and *slim1* 2B groups exposed to B toxicity, the ANAC085 gene emerged as one of the most significantly up-regulated genes, indicating its central role in adapting to B toxicity. Conversely, heat shock proteins, particularly HSP17.6, were among the most down-regulated genes in both *slim1* mutant under 1B and 2B conditions. This suggests challenges in protein stabilization under B toxicity conditions, with the repression of these processes serving as a regulatory strategy for energy conservation during adaptation.

In the expression profile results of genes effective in GSH processes, a decrease in the expression levels of *APX1* and *GPX1* genes was observed. This shows that B toxicity may repress these genes while combating oxidative stress. These genes provide cellular balance by neutralizing ROS. In *slim1* mutants, increased ROS levels due to cellular accumulation of B ions may cause these genes to fail to function adequately. Furthermore, this decrease may be due to disruptions in the glutathione pathway, since enzymes such as *APX1* and *GPX1* are dependent on the presence of glutathione [99].

Although a slight decrease was observed in *GSH1* and *GSH2* genes, their expression levels generally remained stable. A decrease may indicate that glutathione production has slowed down due to B toxicity or that cellular resources have been directed to other defense mechanisms. However, the absence of a serious suppression in these genes suggests that the cell tries to keep glutathione synthesis at critical levels and that glutathione continues to be one of the main defense mechanisms against toxicity. On the other hand, the decrease in the expression levels of the *PCS1* gene may indicate that a phytochelatin-based defense mechanism against B toxicity is not sufficiently activated.

The increase in *GSTU24* ensures that toxic compounds bind with glutathione, making them less harmful and removing them from the cell. This process allows the plant to alleviate the effects of oxidative stress, reduce toxicity and maintain its metabolic balance. Specifically, the increase in *GSTU24* in the *slim1* mutant indicates that the plant has developed a compensation mechanism against deficiencies in sulfur or glutathione metabolism by increasing detoxification mechanisms [100].

PCS1 plays an important role in the detoxification of heavy metals. However, in the analysis results, it was observed that *PCS1* was not functional enough in response to B toxicity. This may indicate that the toxic effects of B ions are being balanced by alternative mechanisms such as glutathione and other secondary metabolites [101].These results reveal that glutathione-based responses to B toxicity are reorganized at the genetic level and that some genes play critical adaptive roles.

Expression changes of genes in anthocyanin biosynthesis pathways reflect adaptive response mechanisms to B toxicity. The increase in the *4CL1* and *C4H* genes suggests increased activation in the early stages of the phenylpropanoid pathway to cope with toxicity. The decrease in the *CHI* gene may indicate a slowdown in the intermediate stages of anthocyanin biosynthesis [102].

The differences in the *DFR* gene indicate that there may be a shift to alternative flavonoid derivatives under toxicity conditions. The fact that the *LDOX (ANS)* gene is severely reduced in *slim1* mutants exposed to B toxicity shows that a critical step in anthocyanin biosynthesis is weakened and the plant limits anthocyanin production under these stress conditions. Additionally, the decrease in the *GSTF12* gene indicates that glutathione transport is impaired under toxicity, and the decrease in the *TT12* and *TT4* genes indicates that there are disruptions in the transport and storage processes of anthocyanins [103]. These changes reveal that cellular priorities against B toxicity are rearranged and energy resources are directed to survival and detoxification. Especially the increase in *4CL1* and *C4H* genes indicates that the activation of the early steps of the phenylpropanoid pathway is increased and priority is given to the management of toxic effects.

PIF3 and *SCL3* genes are indirectly linked to anthocyanin biosynthesis. *PIF3* can bind to the promoter regions of genes that regulate anthocyanin biosynthesis by participating in light signaling pathways. However, this regulatory effect occurs together with the active regulation of the *HY5* transcription factor [104]. *HY5* and *PIF3* positively contribute to anthocyanin biosynthesis and can increase anthocyanin production with environmental signals. The increase in the *PIF3* gene in the graph shows that light signal-dependent regulations become more active as an adaptive response to B toxicity.

The increase in the *SCL3* gene indicates that it keeps growth regulation active through gibberellin signaling and may indirectly affect anthocyanin biosynthesis. The relationship of GRAS transcription factors with flavonoid biosynthesis genes supports this mechanism [105].These changes in *PIF3*, *HY5* and *SCL3* genes reveal that anthocyanin biosynthesis is intricately associated with light signals, energy regulation and growth processes. An increase in the *NIP5;1* gene was observed and this was considered as an adaptive response to the regulation of B ion uptake.

TT8 has been shown to contribute to cellular defense by regulating the promoter regions of anthocyanin production genes, and is involved in cellular processes such as intravacuolar transport and water movements. In particular, it reduces cellular toxicity by promoting the accumulation of flavonoids and other toxic metabolites in the vacuole [106].

Changes in the *TT8* gene decrease compared to the *slim1* control group, but increase compared to the WT. This suggests that *TT8* plays a critical role in the regulation of flavonoid and anthocyanin accumulation and that *slim1* mutants adapt under B toxicity [20]by keeping the expression of this gene above the WT level. The increase in *TT8* shows that flavonoid biosynthesis is partially supported and that these molecules contribute to defense mechanisms in toxicity conditions.

The fact that the expression in the *PAP1* and *PAP2* genes remains stable indicates that the basic steps of anthocyanin biosynthesis are preserved, but show a limited increase due to energy prioritization. In general, the regulation of genes under B toxicity can be considered as an effort to maintain a balance between growth, energy and defense processes. These results indicate that genes contributing directly or indirectly to anthocyanin biosynthesis are complexly regulated under stress conditions.

Under the effect of B toxicity, it is shown that there are important changes in the genes related to GSL biosynthesis in the adaptation process of plants to stress conditions. As one of these changes, the stability of genes such as *MAML-4, MYB28 and FMO GS-OX2* can be shown. This stabilization shows that it is protected in order to support the basic steps of biosynthesis under toxic conditions. As one of the important points, the stability of transcription factors such as *MYB28*, which is among the main regulators of GSL biosynthesis, is an important indicator that biosynthesis processes do not stop completely despite the presence of B toxicity [107]. [20] [20]

On the other hand, especially in the 2B condition of *slim1* mutants, a significant suppression is observed in genes such as *BCAT4, CYP79F1, CYP79F2*, and *SOT (SOT16, SOT17, SOT18)* compared to the WT control. This indicates that B toxicity is effective in critical steps such as both side chain extension (BCAT4) and sulfation (SOT) in GSL biosynthesis. The suppression in these genes can be explained by the diversion of cellular resources to combat toxicity and the decrease in the priority order of GSL biosynthesis [108].

Furthermore, the decrease in genes involved in aliphatic GSL production, such as *CYP79F1* and *CYP79F2*, indicates that plants reorganize metabolic processes and save energy under toxicity conditions. In addition, the suppression of *MYB29*, one of the regulatory genes, suggests a decrease in the general regulation of GSL biosynthesis. The more pronounced suppression observed in the 2B condition of *slim1* mutants indicates that stress is felt more intensely and biosynthesis processes are more restricted under these conditions. This suggests that the *slim1* gene may play a critical role in defense responses against B toxicity [109].

In conclusion, changes in GSL biosynthesis genes clearly demonstrate the capacity of B toxicity to regulate metabolic processes. While genes that remain stable (MAML-4, MYB28, FMO GS-OX2) indicate that the basic components of biosynthesis are preserved, suppression in other genes indicates that metabolic priorities under stress are rearranged and resources are directed to defense mechanisms. The increased suppression observed in *slim1* mutants in the 2B condition clearly demonstrates the role of genetic regulation in defense responses to B toxicity.Changes observed in the expression levels of GSL transport and modification genes under B toxicity indicate stress response mechanisms and adaptive processes in plants.

The decrease in *AOP1*, *GSTF9* and *GSTU20* genes suggests that B toxicity suppresses GSL modification processes and these processes are not prioritized under toxic conditions. In particular, *GSTF9* and *GSTU20* genes are known to play important roles in glucosinolate binding and transport, and the decrease in these genes indicates that detoxification mechanisms are limited [108].

The increase in the SULTR1;2 gene indicates that sulfur transport is increased as an adaptive response to B toxicity. This increase in sulfur transport may be important for the maintenance of GSL biosynthesis or the production of sulfur-containing compounds during detoxification processes [110]. This increase can be evaluated as the plant's effort to produce protective metabolites against B toxicity. The decreases observed in *SULTR2;1* and *SUR1* genes suggest that B toxicity leads to restriction of sulfur transport and glucosinolate biosynthesis processes. These genes are important for GSL metabolism and transport. The suppression of these genes under 1B and 2B conditions suggests that energy and resources may be directed to other cellular defense processes under toxicity [111].

In conclusion, upregulation of GSL transport and modification genes under B toxicity indicates that the plant restructures its resources and prioritizes defense mechanisms against toxicity. The increase in the *SULTR1;2* gene indicates that sulfur transport against B stress is increased and the plant's defense capacity is strengthened. In contrast, repression of the *SULTR2;1* and *SUR1* genes reflects an adaptation in these processes to save energy and direct resources to other defense mechanisms. These different genetic responses to B toxicity reveal that the plant has developed a multilayered adaptation mechanism to combat stress.

Expression changes of genes related to GSL metabolism and catabolism under B toxicity provide critical information for understanding the adaptive defense mechanisms and energy management of plants. The increase in *APK1A*, *APK2A*, and *APK2B* genes indicates that sulfation mechanisms become more active under conditions of toxicity. The role of these genes in sulfation processes is critical for the modification and stabilization of glucosinolates [108]. The increase suggests that defense mechanisms against B stress are an adaptation to maintain glucosinolate biosynthesis.

The expression levels of *APS1* and *APS2* genes close to the WT control group suggest that sulfur assimilation is kept constant under toxicity conditions and these processes are regulated to meet the metabolic requirements of the plant. Sulfur is critically important in both GSL biosynthesis and other detoxification processes [110].

The increase in the *BGLU28* gene suggests that glucosinolate catabolism is accelerated under toxicity conditions and that this operates as part of defense responses. The products of this gene are involved in the breakdown of glucosinolates and the formation of toxic byproducts. Against B toxicity, this process may be an important mechanism to alleviate toxic effects and protect the plant against environmental stresses [112]. The slight decrease seen in *GGP1* and *GGP3* genes may indicate that energy and resources in glucosinolate metabolism are being diverted to other critical processes. B toxicity can trigger the plant to restructure its defense metabolism towards energy efficiency [108].

Generally evaluated, it can be said that B toxicity reshapes defense mechanisms by affecting glucosinolate metabolism in both protective and limiting aspects. The increase in *APK1A*, *APK2A*, *APK2B*, and *BGLU28* genes indicates that adaptive responses to B toxicity are regulated through glucosinolate modification and catabolism. In contrast, the slight reduction in *GGP1* and *GGP3* genes may be associated with prioritization of resources under

toxicity. These results demonstrate that glucosinolate metabolism provides a flexible and dynamic response to B toxicity.

The gene expression responses of *slim1*1B and 2B mutants to B toxicity exhibit different adaptive strategies and regulations specific to toxic conditions. Genes such as *PCR1*, *CYP79F1* and *CYP79F2* were upregulated in both conditions. These genes exhibit common defense mechanisms such as maintenance of metal ion homeostasis and enhancement of chemical defense by increasing glucosinolate biosynthesis.

In addition, the *IAA5* gene regulates auxin signaling to support the persistence of growth process in both conditions. The main differences between 1B and 2B conditions are related to stress response and maintenance of cell structure. In condition 1B, stress response and immune-related genes such as *AIG1* and *ELI3-2* were upregulated. This shows that the less toxic B is, the more the immune response is at the forefront.

In both cases, it was observed that when genes were suppressed, transport and metabolic processes were restricted. For example, genes related to transport and ribosomal processes such as ABCB12 and AT1G12030 were suppressed in conditions 1B. Similarly, in conditions 2B, metabolite movement and metabolic processes were restricted by repressing the genes ABCB12 and AT1G08117.

6. CONCLUSION

In this study, gene expression profiles of *slim1* mutants were analyzed under control and B toxicity conditions and significant regulation was detected in sulfur metabolism, secondary metabolite biosynthesis and stress response pathways. Analysis in *Arabidopsis thaliana* leaf tissues revealed how metabolic processes and cellular responses were re-regulated against B toxicity. In particular, sulfur transport and metabolism, binding activities and protein stabilization pathways were observed to be regulated under B toxicity.

In the study, it was found that the LSU1 gene was up-regulated in all conditions. This gene, which is a key regulator of sulfur metabolism, plays a critical role in maintaining sulfur homeostasis. In *slim1* 1B and *slim1* 2B mutants exposed to B toxicity, the ANAC085 transcription factor was found to be among the most up-regulated genes. The up-regulation of ANAC085 indicates that cellular defense mechanisms and adaptation strategies against B toxicity are actively involved. Up-regulation of processes such as ribosome biogenesis and RNA processing in the *slim1* 1B mutant contributes to stress adaptation by increasing cellular protein production.

In contrast, suppression of heat shock proteins such as HSP17.6 was noted in both mutants. Heat shock proteins generally play important roles in protein folding and stability. The suppression of these genes leads to difficulties in protein stabilization, suggesting that cellular stability is limited. Furthermore, genes associated with binding activities are upregulated in the *slim1* 2B mutant, indicating that this mutant has developed a more focused energy-saving and genetic regulation strategy.

GO and KEGG analyses showed upregulation of essential processes such as ribosome biogenesis, binding activities and sulfur metabolism under B toxicity conditions. Activation of energy-intensive processes (e.g. ribosome biogenesis and RNA processing) in the *slim1* 1B mutant indicated that adaptive responses were regulated in a wide range. In the *slim1* 2B mutant, binding activities were more specifically increased, and energy-intensive processes such as protein stabilization were partially suppressed. KEGG mapping analyses revealed adaptive upregulation of flavonoid biosynthesis pathways under B toxicity. In particular, the up-regulation of genes that increase the production of compounds such as

kaempferol and quercetin indicated that flavonoids play a critical role in defense against B toxicity.

In conclusion, genetic modifications developed by *slim1* mutants against B toxicity revealed both common and genotype-specific adaptation strategies in plants. This study showed that flavonoid biosynthesis, sulfur metabolism, and protein stabilization should be evaluated as genetic modification targets for B toxicity-resistant plant development strategies. Future detailed analyses will allow us to understand the molecular mechanisms of these adaptation processes in more depth.

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